Table 1

Immunization of rabbits and rhesus monkeys with anti-id BR55-2 #E4 or unspecific mouse IgG1

Relative titer increase at week 9 compared to 1:4 diluted preserum (Cell ELISA)

	Rabbit		Monkey	
	anti-id BR55-2	mouse IgG1	anti-id BR55-2	mouse IgG1
SKBR5 (Y+)	1000	16	1000	8
WM9 (Y-)	3	n.t.	25	n.t.

0502E0 E5000001

Table 2

Boost immunization of rhesus monkeys with anti-Id BR55-2 #E4 or unspecific mouse IgG1

Binding of monkey serum Ig to a SKBR5-membrane preparation (ELISA)
Relative titer increase compared to 1:4 diluted preserum

	9 weeks after first course	2 years after first course	1 week after first boost	4 weeks after first boost
anti-Id BR55-2	40	1	80	50
mIgG1	1	2	n.t.	2

2050607 5906067

Table 3

Binding of rhesus monkey's serum Ig to HIV-infected/non-infected PALL-cell line (indirect immunofluorescence, IFA-Anti-HIV1-Kit Waldheim)

	Immunization of rhesus monkeys with anti-id BR55-2 #E4		unspecific mouse-IgG1		Human serum controls (Kit)	
PALL:	HIV+	HIV-	HIV+	HIV-	PALL: HIV+	HIV-
preserum					**HIV-neg. human serum**	
conc.	1	1	1	1	0	0
1:10	1	1	1	1		
serum 9 weeks after first immunziation					**HIV-pos. human serum**	
conc.	3	1	1-2	1	5	0
1:10	3	1	1-2	1		

0-5 = intensity of fluorescence (anti-human IgG-FITC)
0 = no fluorescence
5 = maximum fluorescence

Table 4

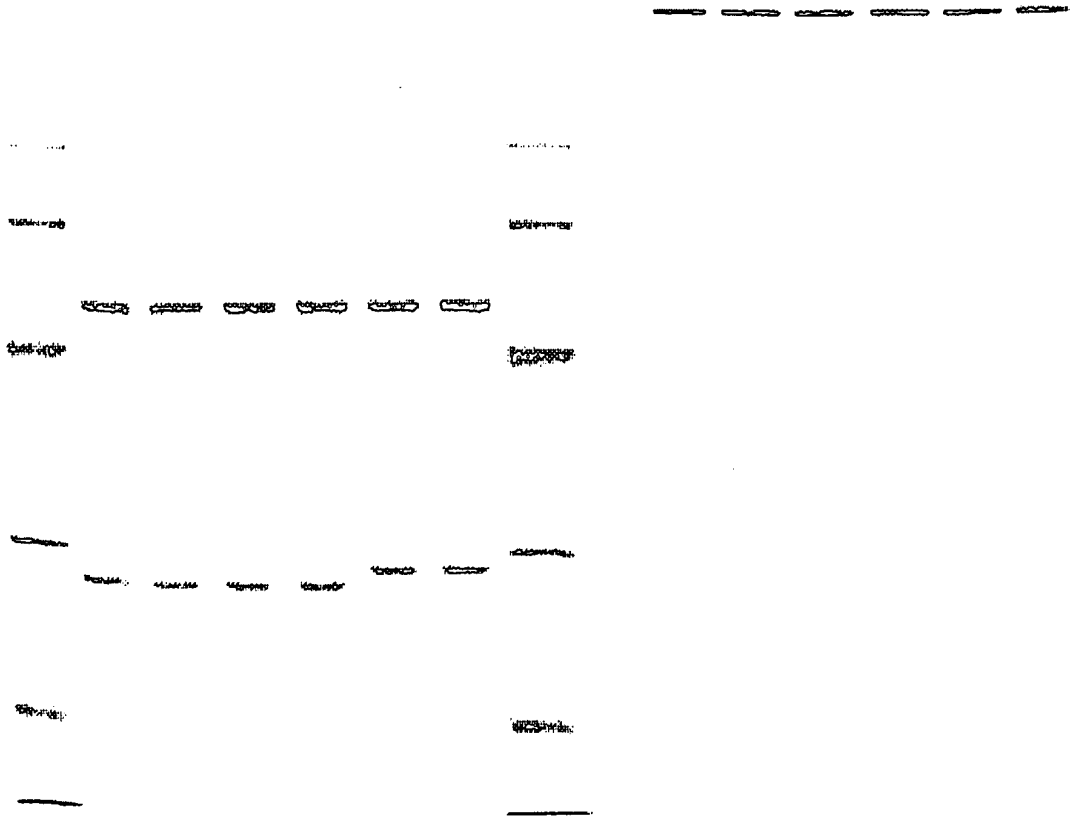
Binding of rhesus monkey Ig immunopurified on anti-id BR55-2 #E4 (AB3) and BR55-2 / murine IgG3 (AB1) to HIV-infected/non-infected PALL-cell line (Indirect immunofluorescence, IFA-Anti-HIV1-Kit Waldheim)

	AB3 (20 µg/ml)		BR55-2 / murine IgG3 (20 µg/ml)	
PALL:	HIV+	HIV-	HIV+	HIV-
	3	0	2	0

	Human serum controls (Kit)	
PALL:	HIV+	HIV-
HIV-neg. human serum	0	0
HIV-pos. human serum	5	1

0-5 = intensity of fluorescence (anti-human IgG-FITC)
0 = no fluorescence
5 = maximum fluorescence

Figure 1
SDS - PAGE

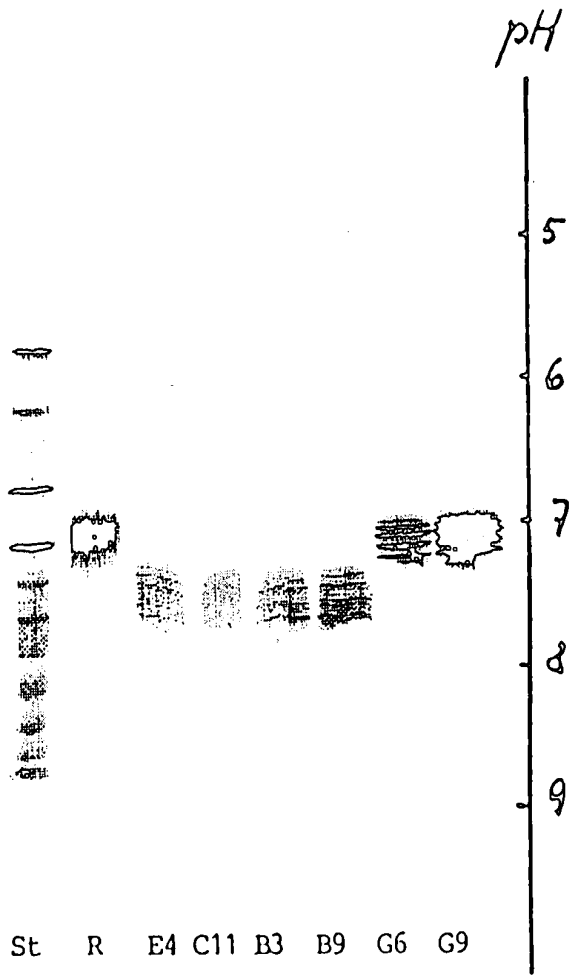


St = Standards

E4, C11, B3, B9, G6, G9 = anti-id BR55-2 Mabs

2030260 E330600T

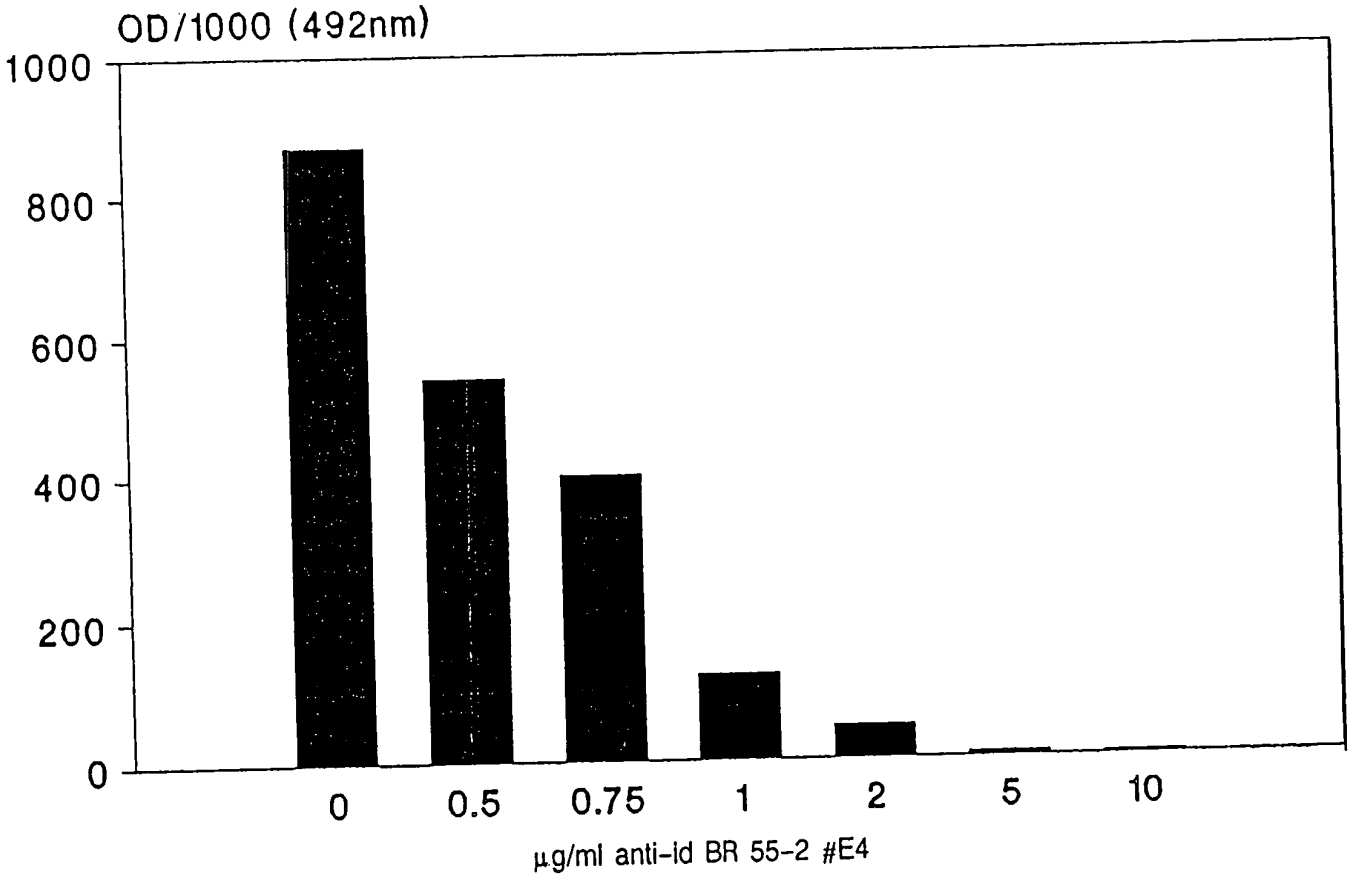
Figure 2
Isoelectric Focussing



St = Standard
R = Reference Mab
E4, C11, B3, B9, G6 and G9 = anti-id BR55-2 Mabs

EUROPEAN PATENT

Figure 3
Binding of BR55-2/murine IgG3 to SKBR5 cell line
Inhibition by anti-id BR55-2 #E4



BR 55-2/murine IgG3: 1 µg/ml

Figure 4
Binding of rabbit Ig to SKBR5 before and after immunization with anti-id BR55-2 #E4 (Cell-ELISA)

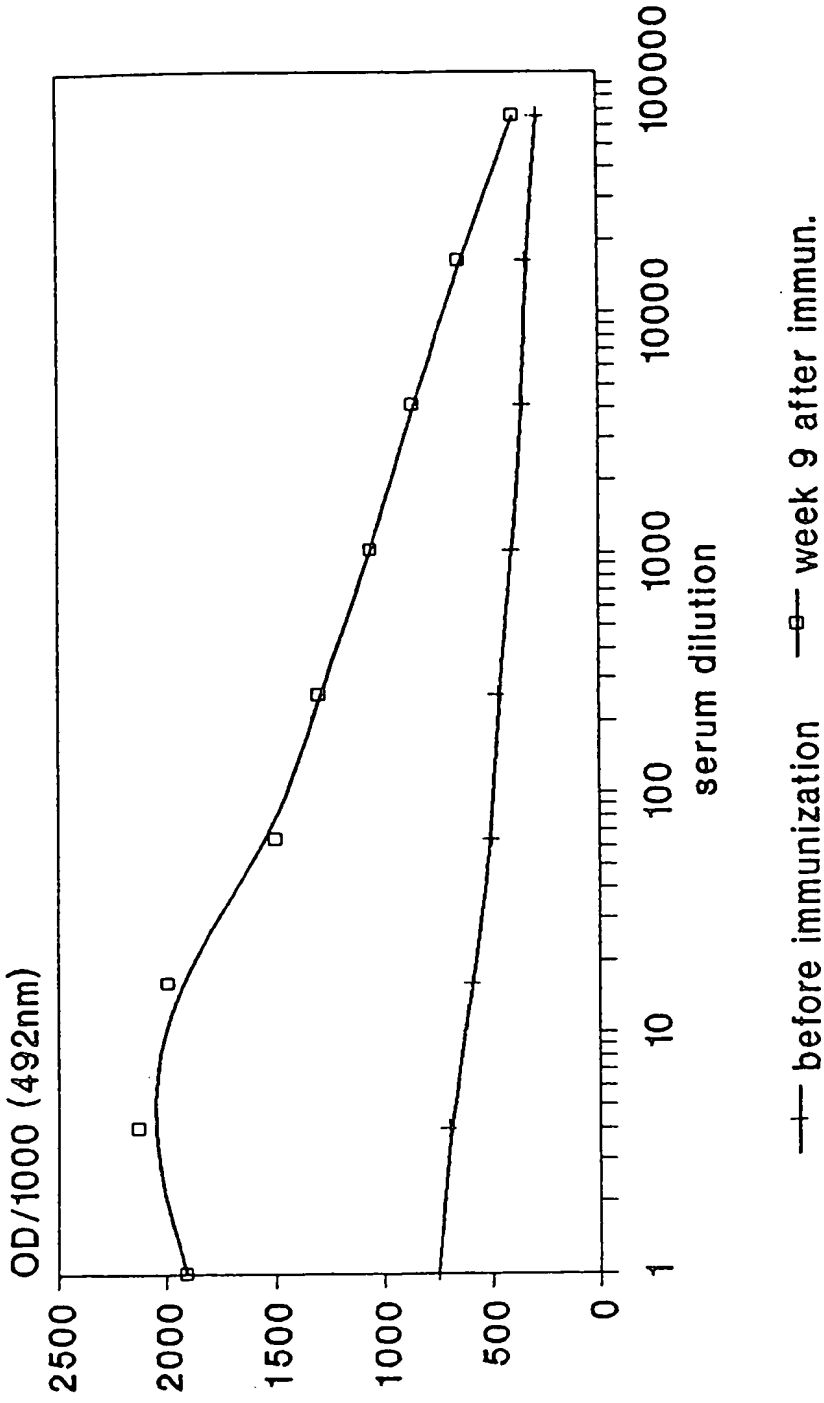
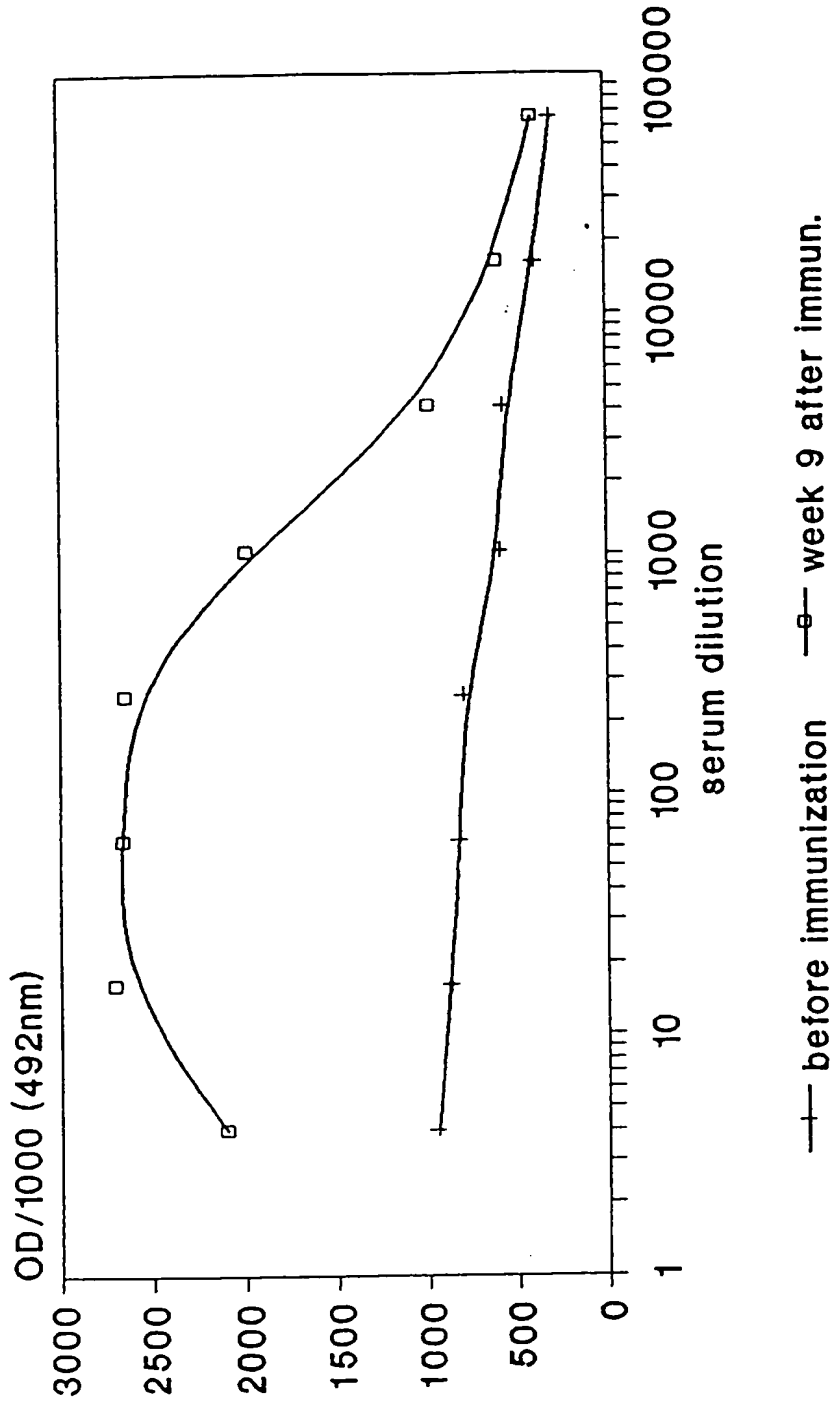


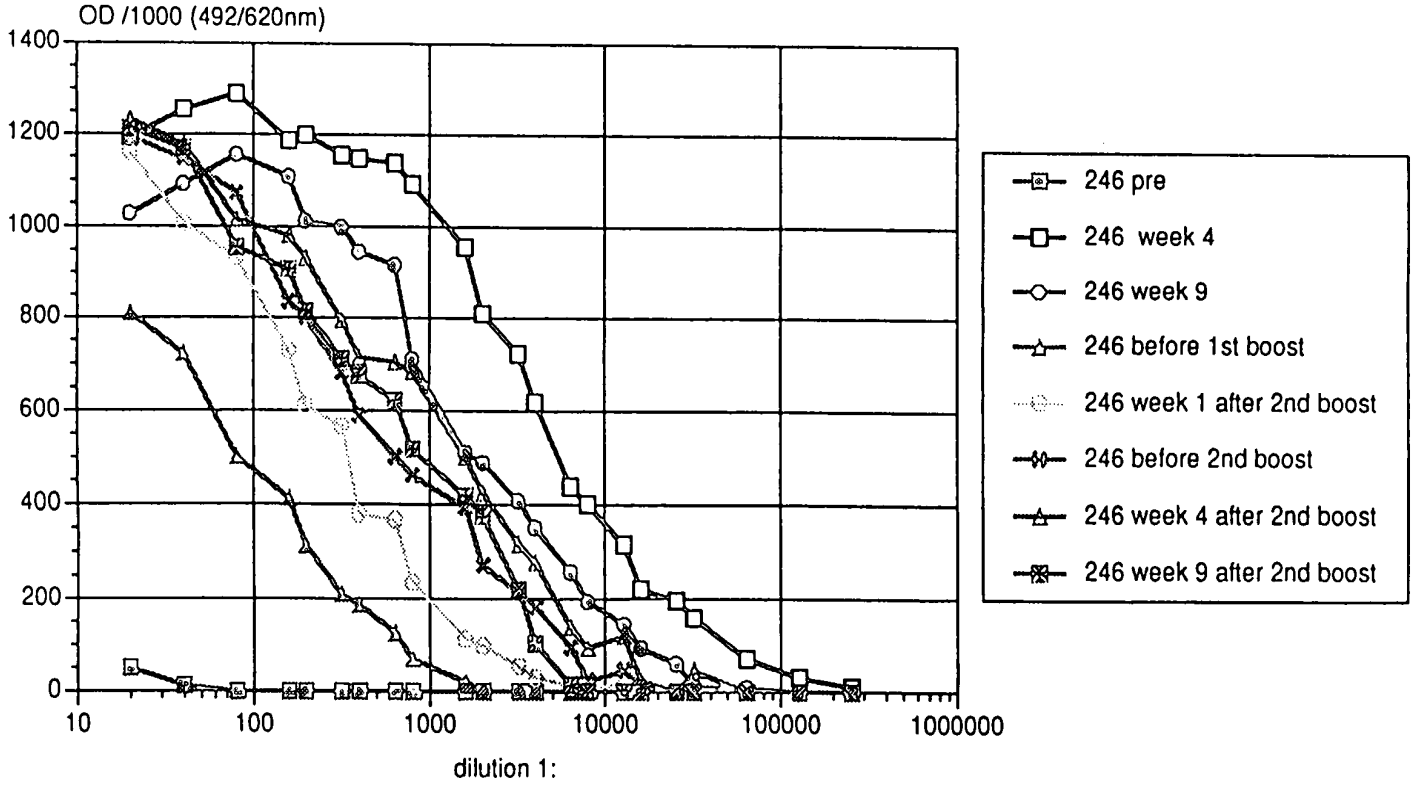
Figure 5
Binding of rhesus monkey Ig to SKBR5 before and after immunization with anti-id BR55-2 #E4 (Cell-ELISA)



SUBSTITUTE SHEET

Figure 6

Immunization of monkey 246 with anti-id BR55-2#E4
Serum Ig binding to anti-id BR55-2#E4
(ELISA)



EP 0 590 607 A1

Figure 7

Immunization of monkey 287 with anti-id BR55-2#E4 Serum Ig binding to anti-id BR55-2#E4 (ELISA)

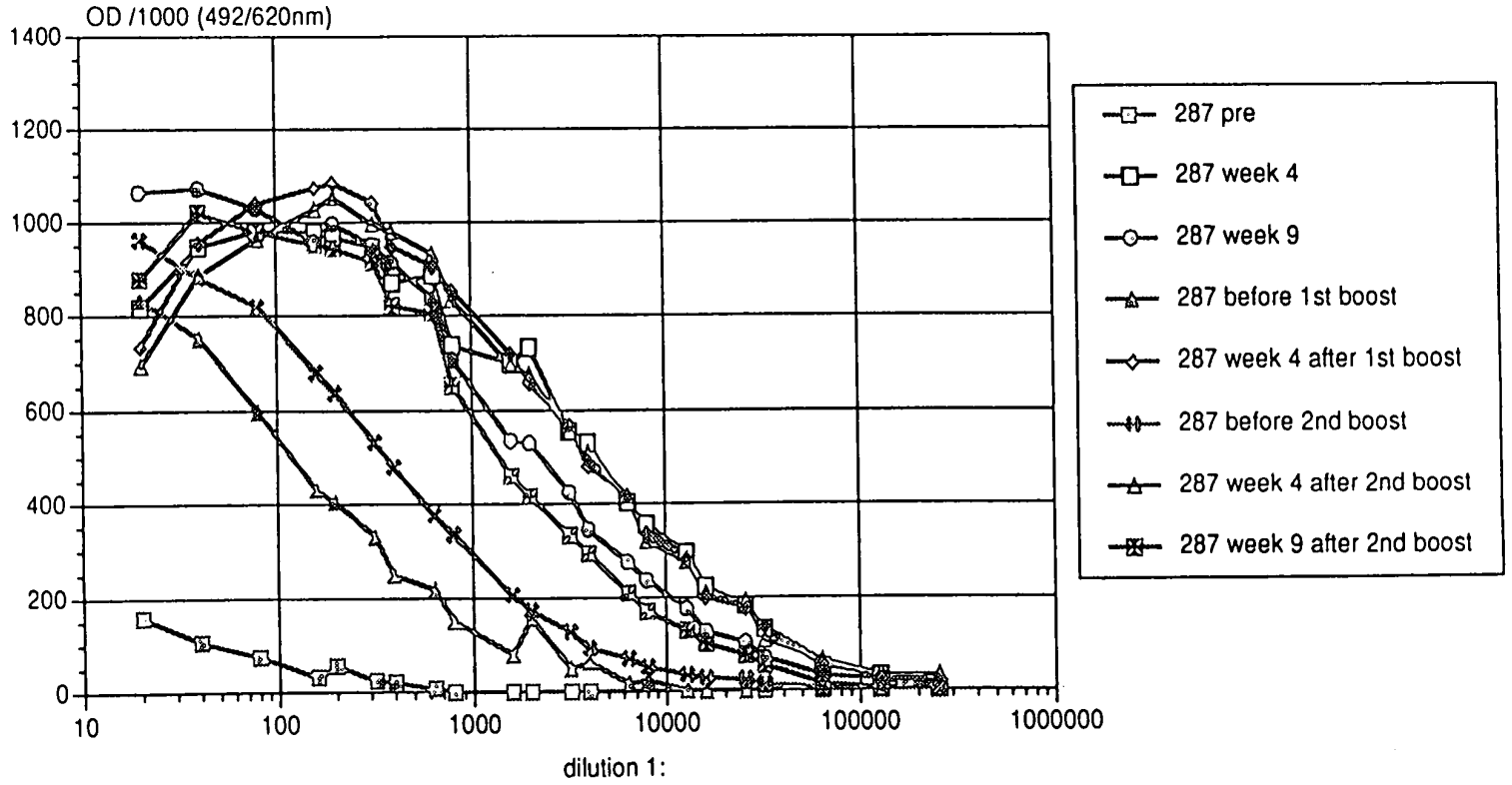


Figure 8

Immunization of monkey E23 with anti-id BR55-2#E4 Serum Ig binding to anti-id BR55-2#E4 (ELISA)

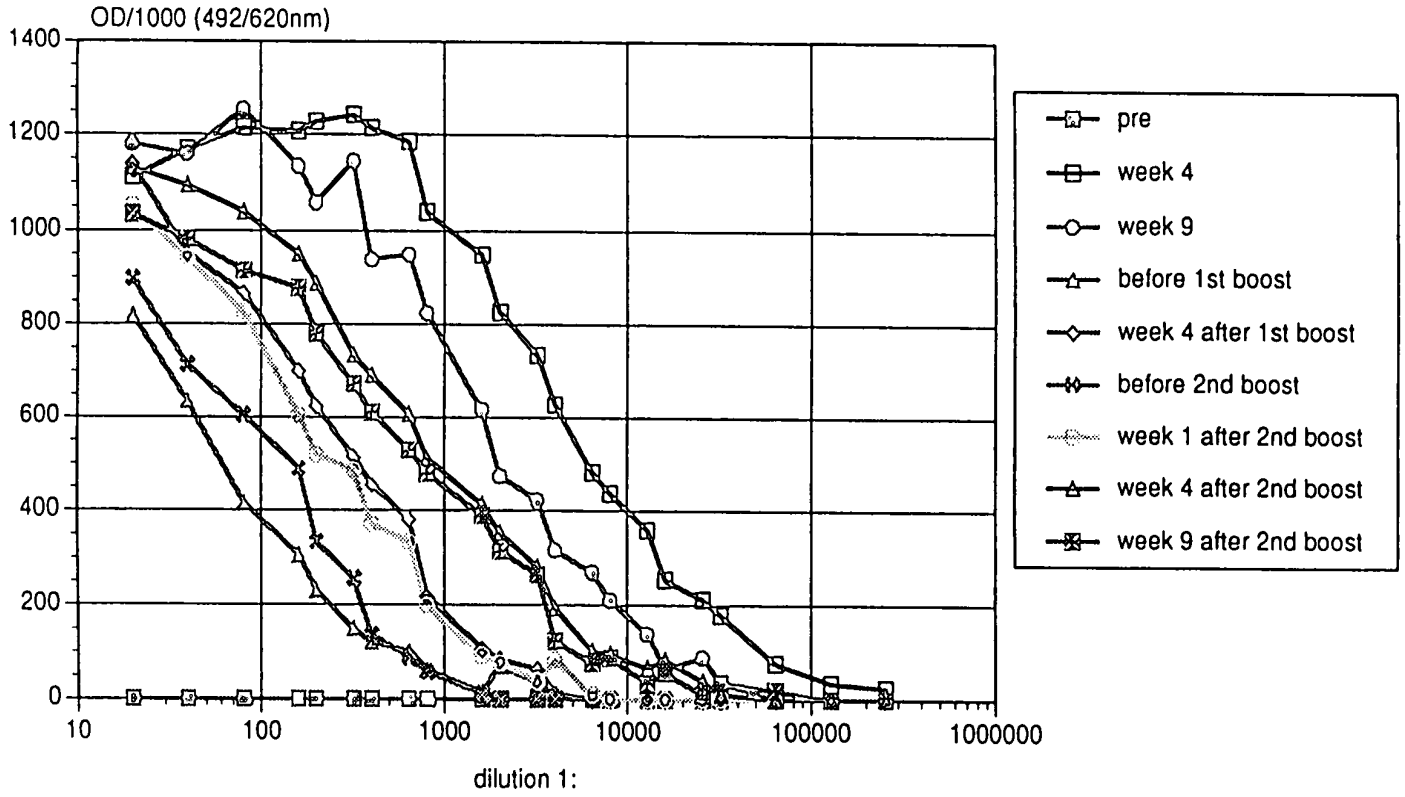


Figure 9

Immunization of monkey 215 with irrelevant mouse IgG1
Serum Ig binding to anti-id BR55-2#E4 (ELISA)

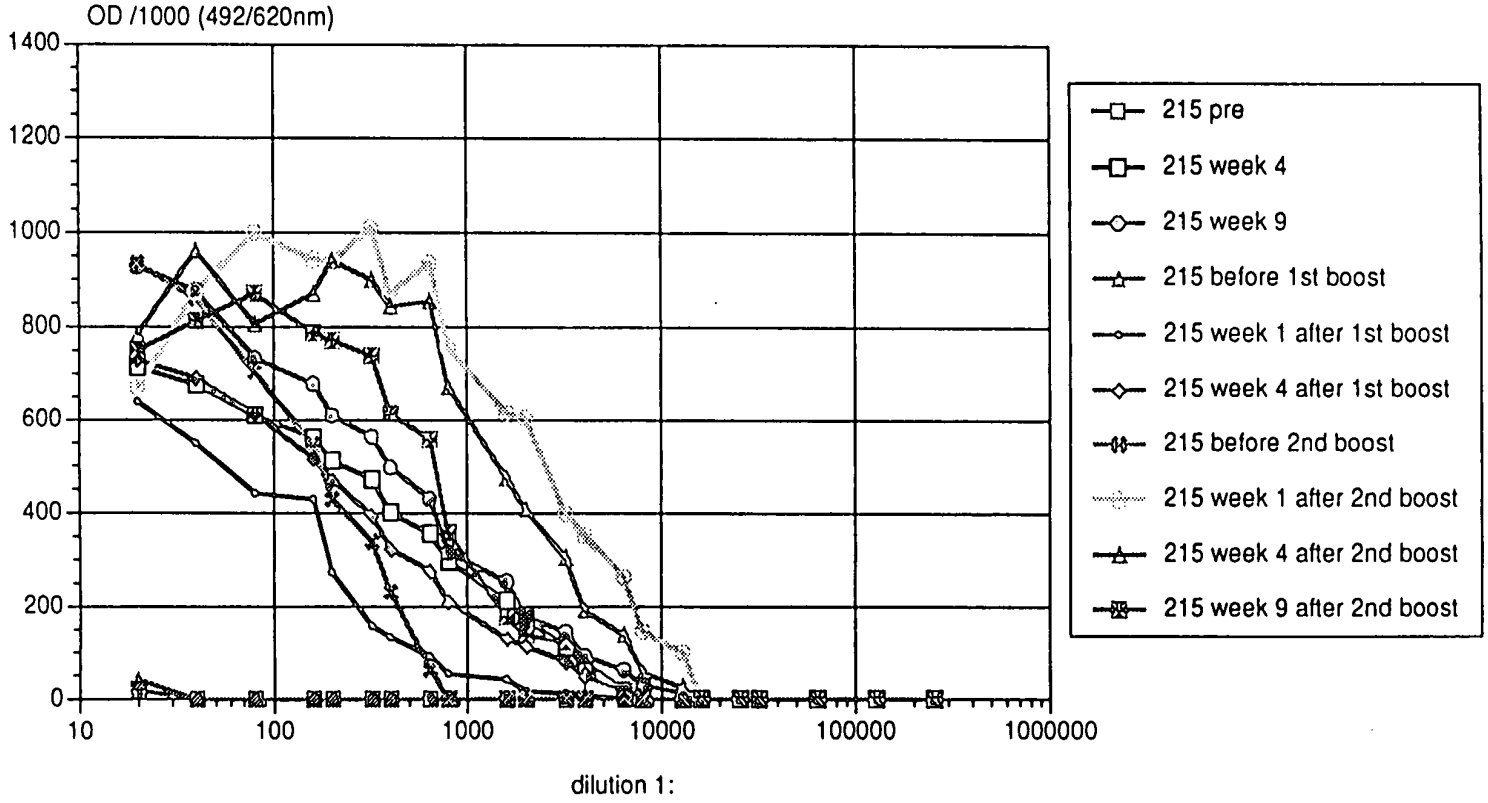


Figure 10

Immunization of monkey 252 with irrelevant mouse IgG1
Serum Ig binding to anti-id BR55-2#E4 (ELISA)

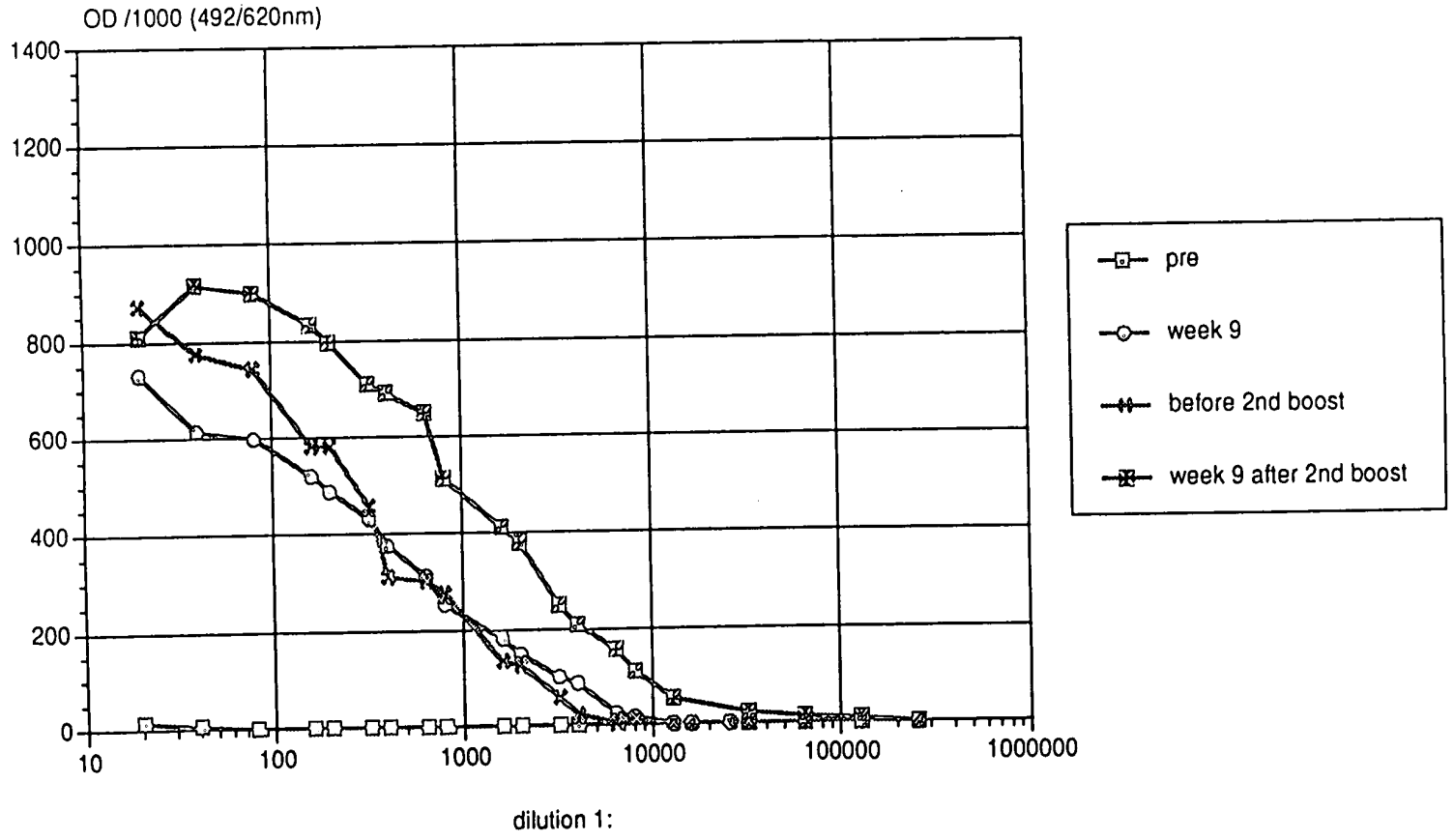


Figure 11

Immunization of monkey 246 with anti-id BR55-2#E4 Serum Ig binding to SKBR5 breast cancer cells (cell- ELISA)

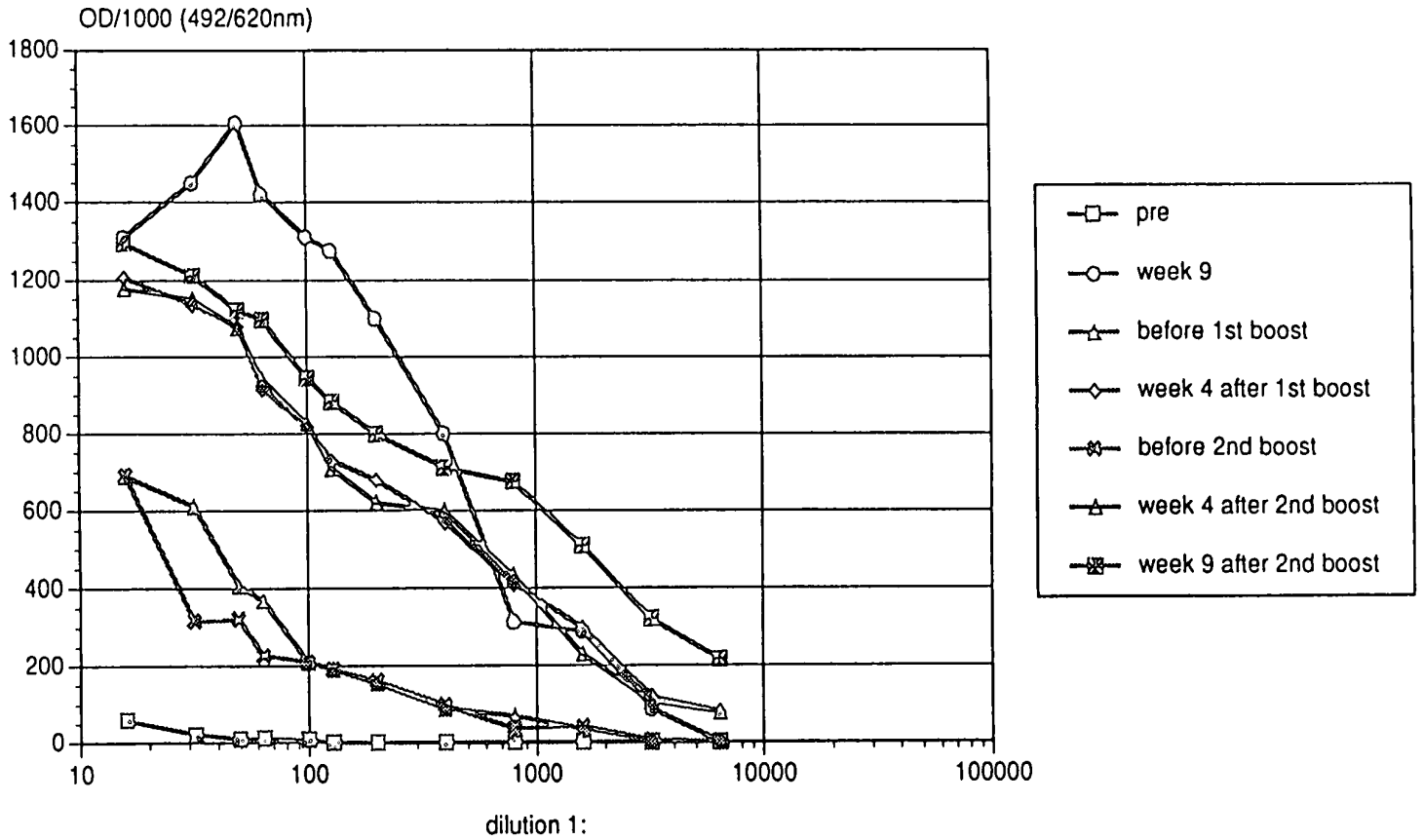


Figure 12

Immunization of monkey 287 with anti-id BR55-2#E4 Serum Ig binding to SKBR5 breast cancer cells (cell- ELISA)

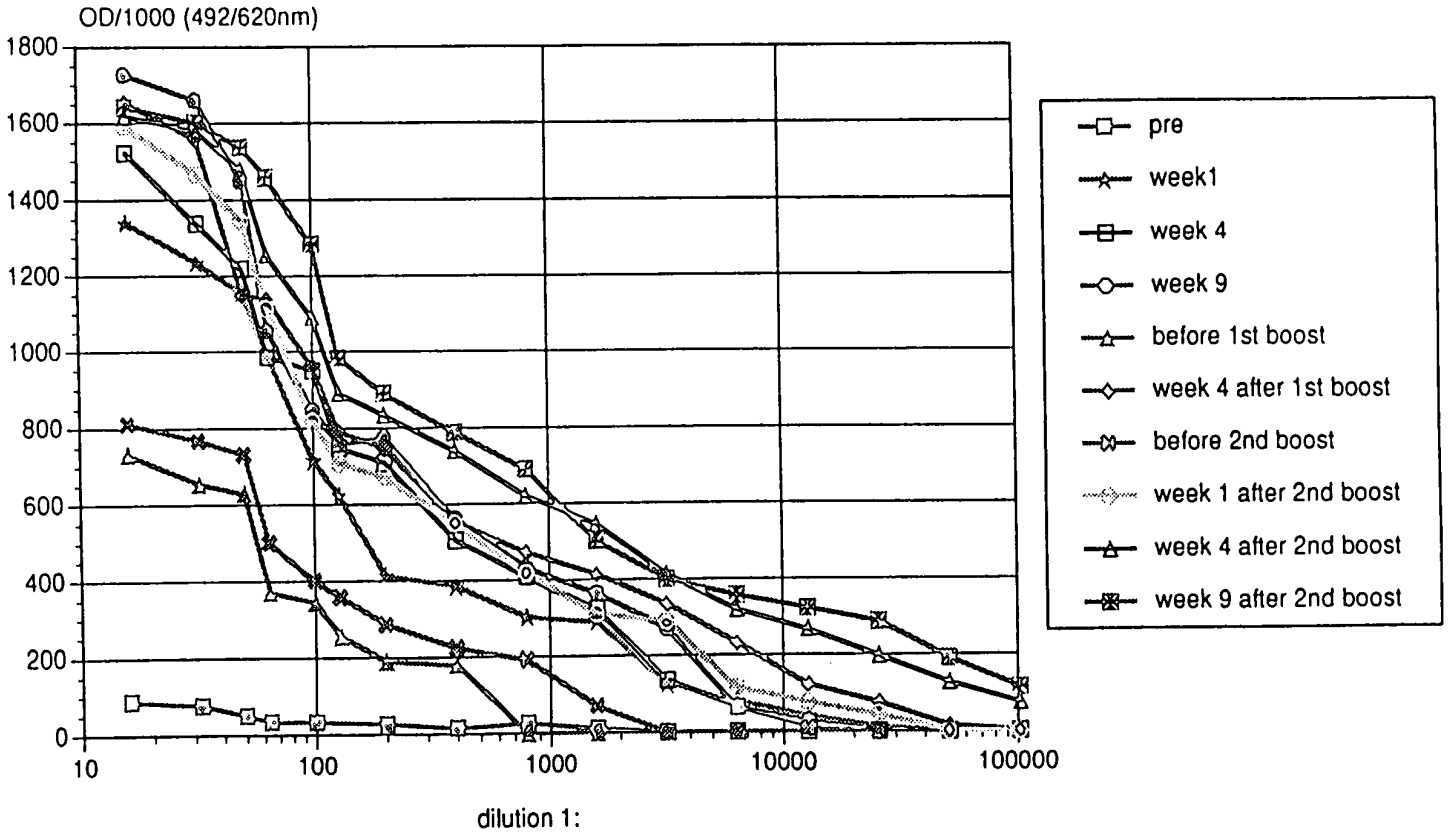


Figure 13

Immunization of monkey E23 with anti-id BR55-2#E4 Serum Ig binding to SKBR5 breast cancer cells (cell- ELISA)

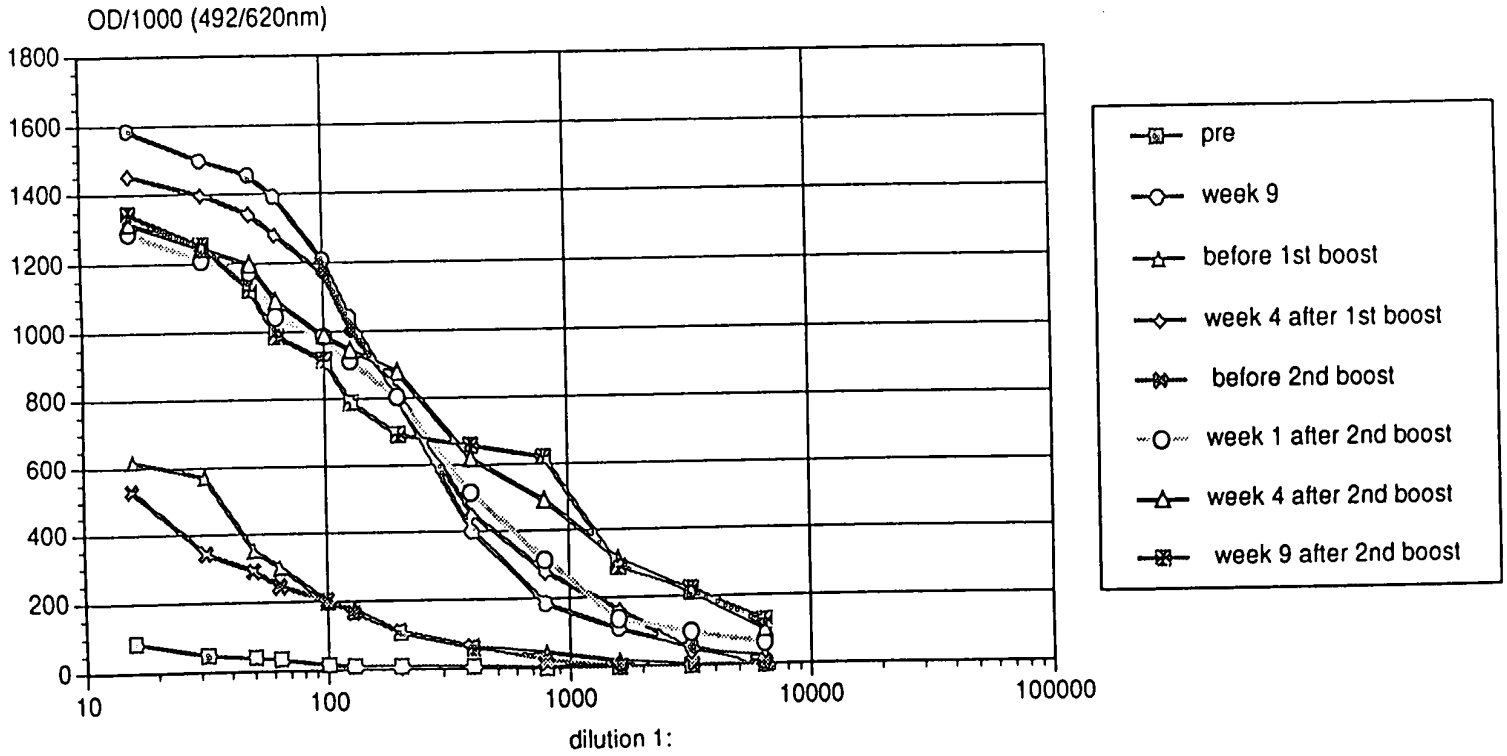


Figure 14

Immunization of monkey 215 with irrelevant mouse IgG1
Serum Ig binding to SKBR5 breast cancer cells
(cell- ELISA)

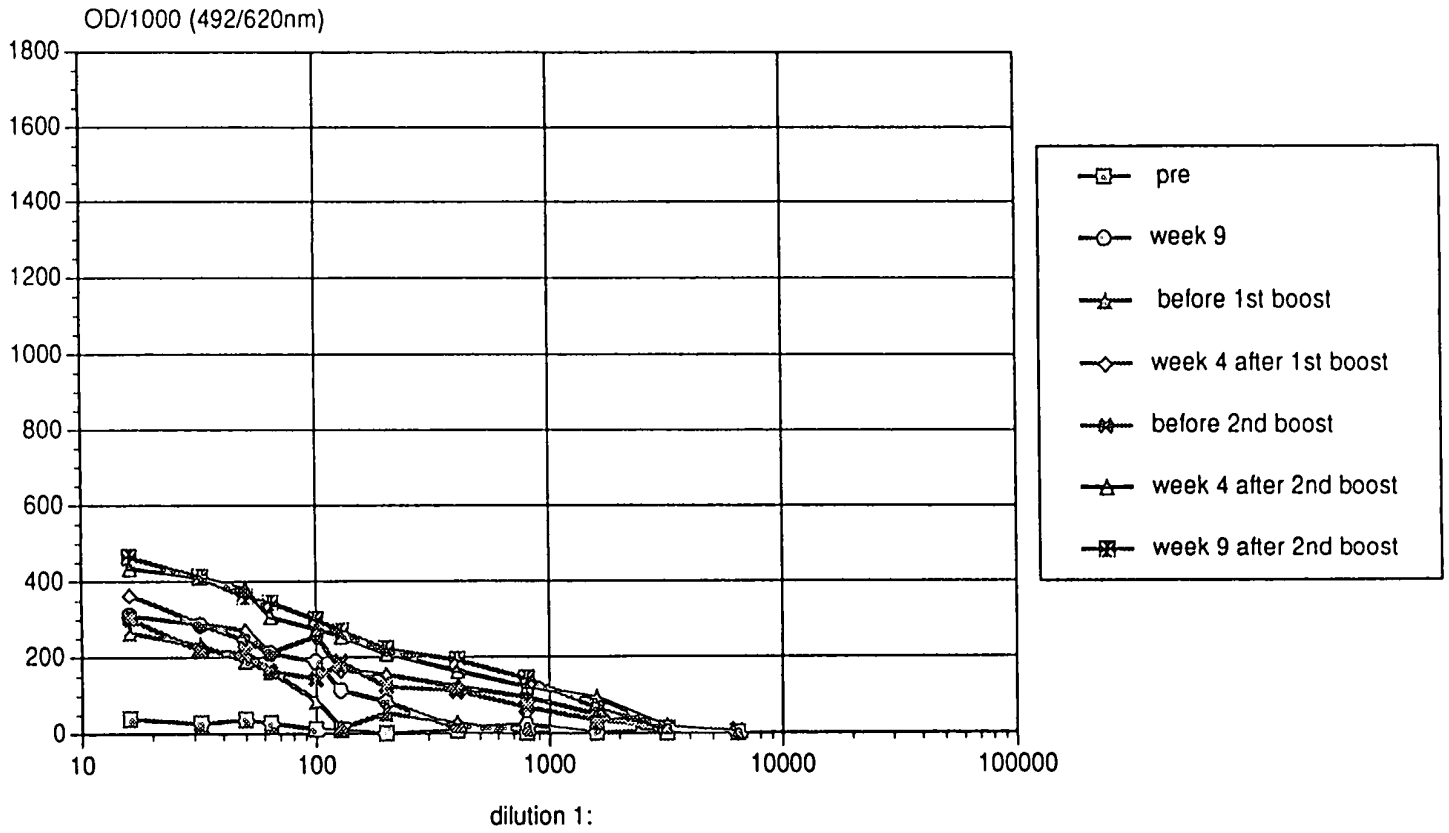


Figure 15

Immunization of monkey 246 with anti-id BR55-2#E4 Serum Ig binding to CATO gastric cancer cells (cell- ELISA)

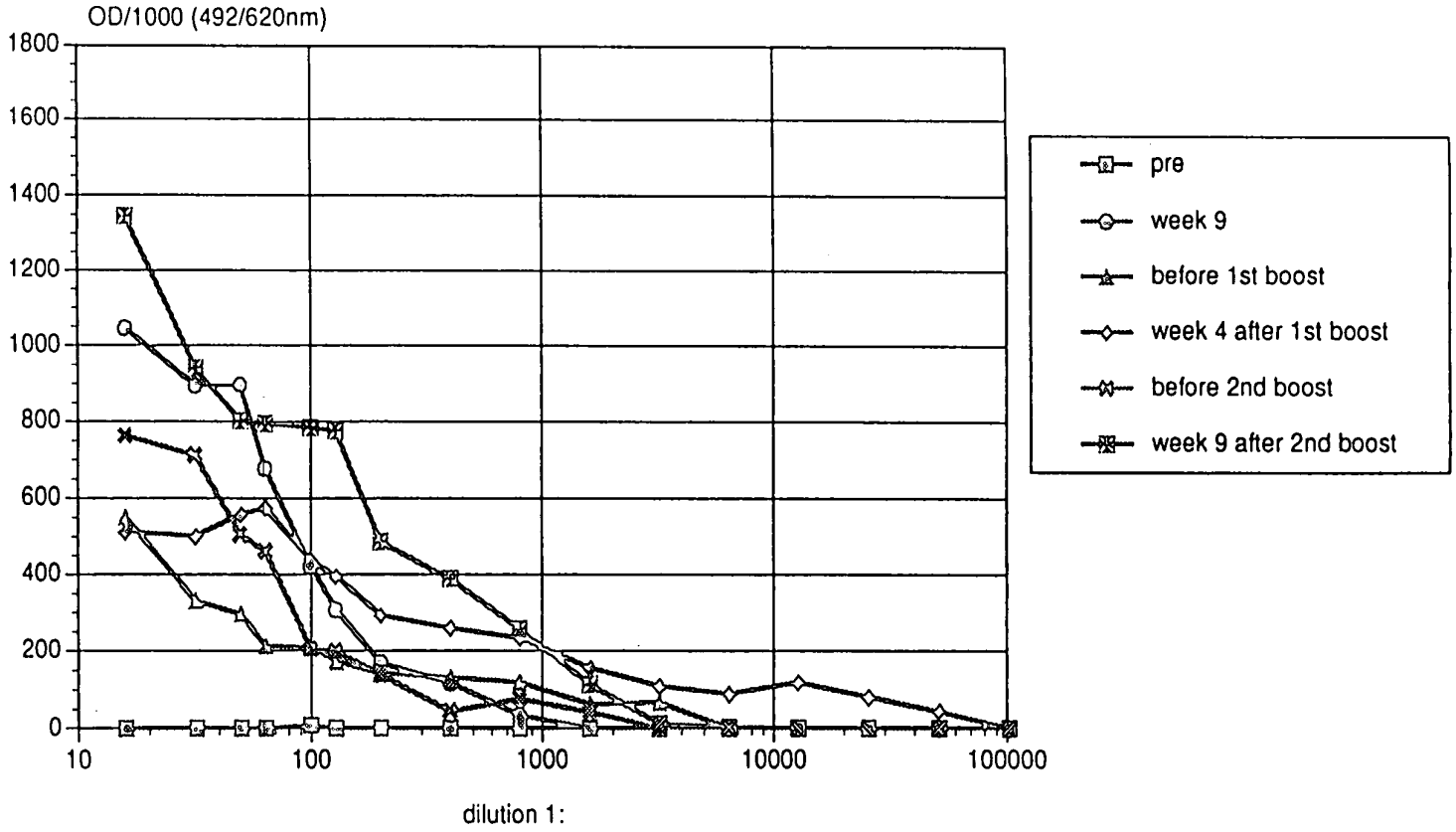


Figure 16

Immunization of monkey 215 with irrelevant mouse IgG1
Serum Ig binding to CATO gastric cancer cells
(cell-ELISA)

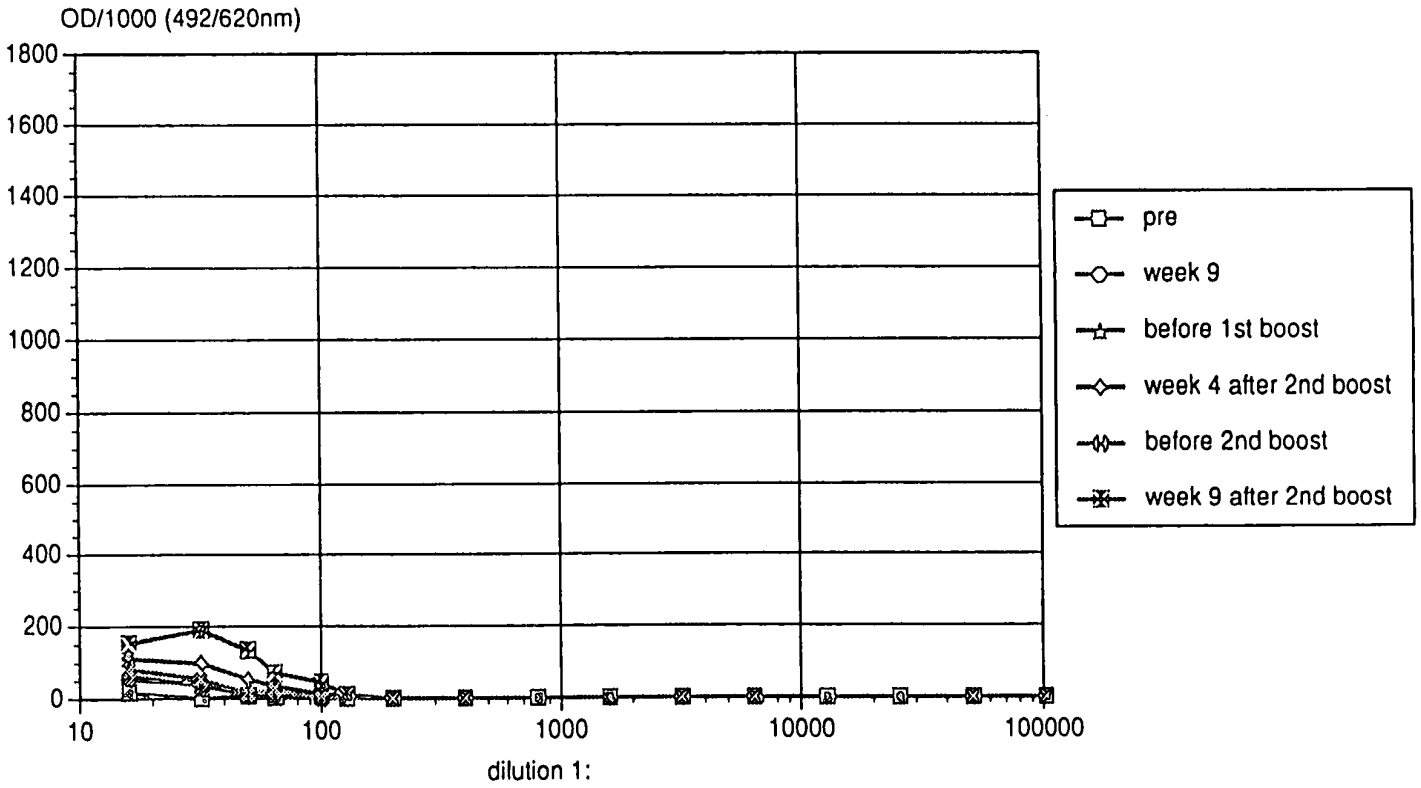


Figure 17

Immunization of monkey E 23 with anti-id BR55-2#E4 Serum Ig binding to SW2 small cell lung cancer cells (cell- ELISA)

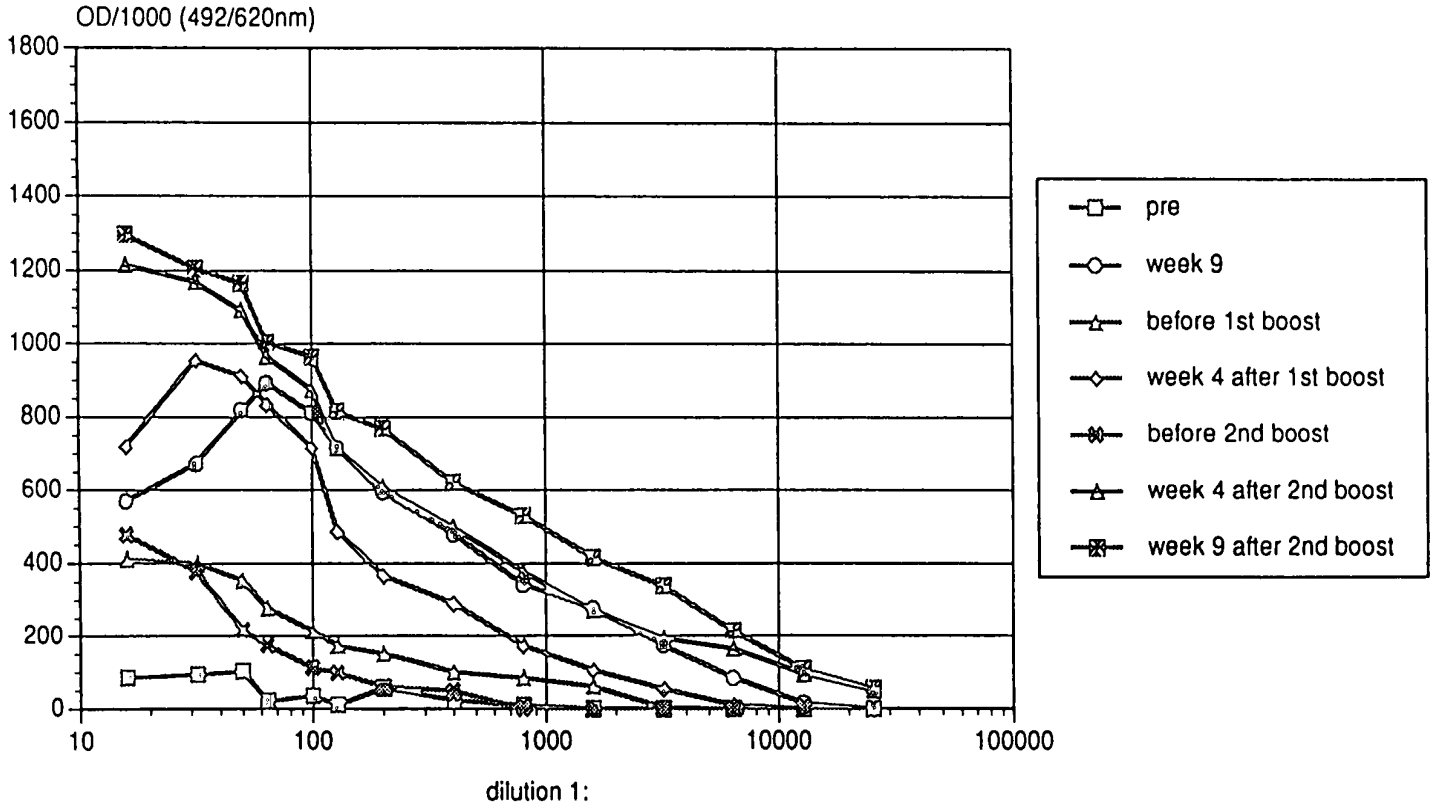


Figure 18

Immunization of monkey 215 with irrelevant mouse IgG1 Serum Ig binding to SW2 small cell lung cancer cells (cell- ELISA)

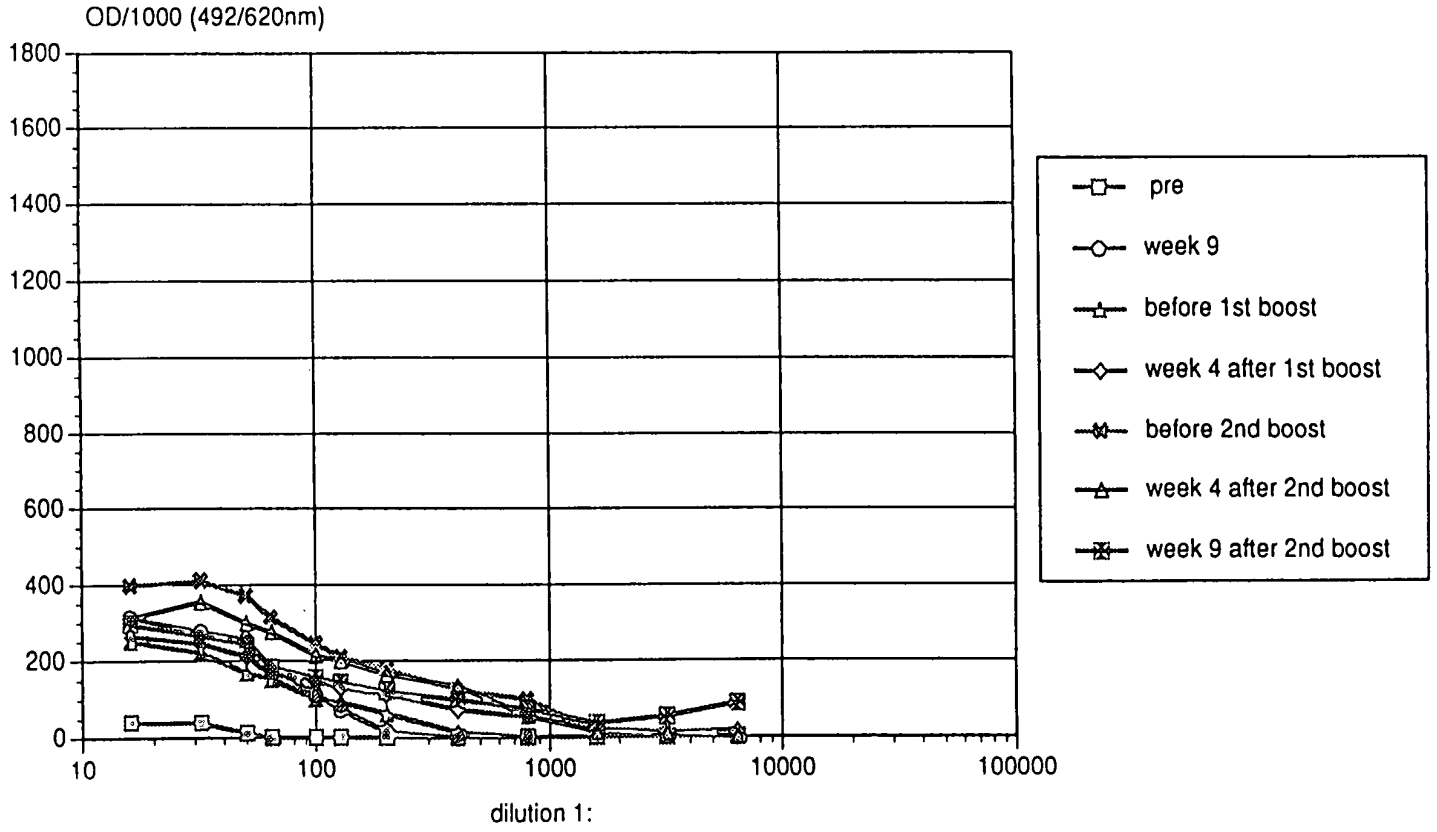


Figure 19

Immunization of monkey E 23 with anti-id BR55-2#E4 Serum Ig binding to SW 948 colon cancer cells (cell- ELISA)

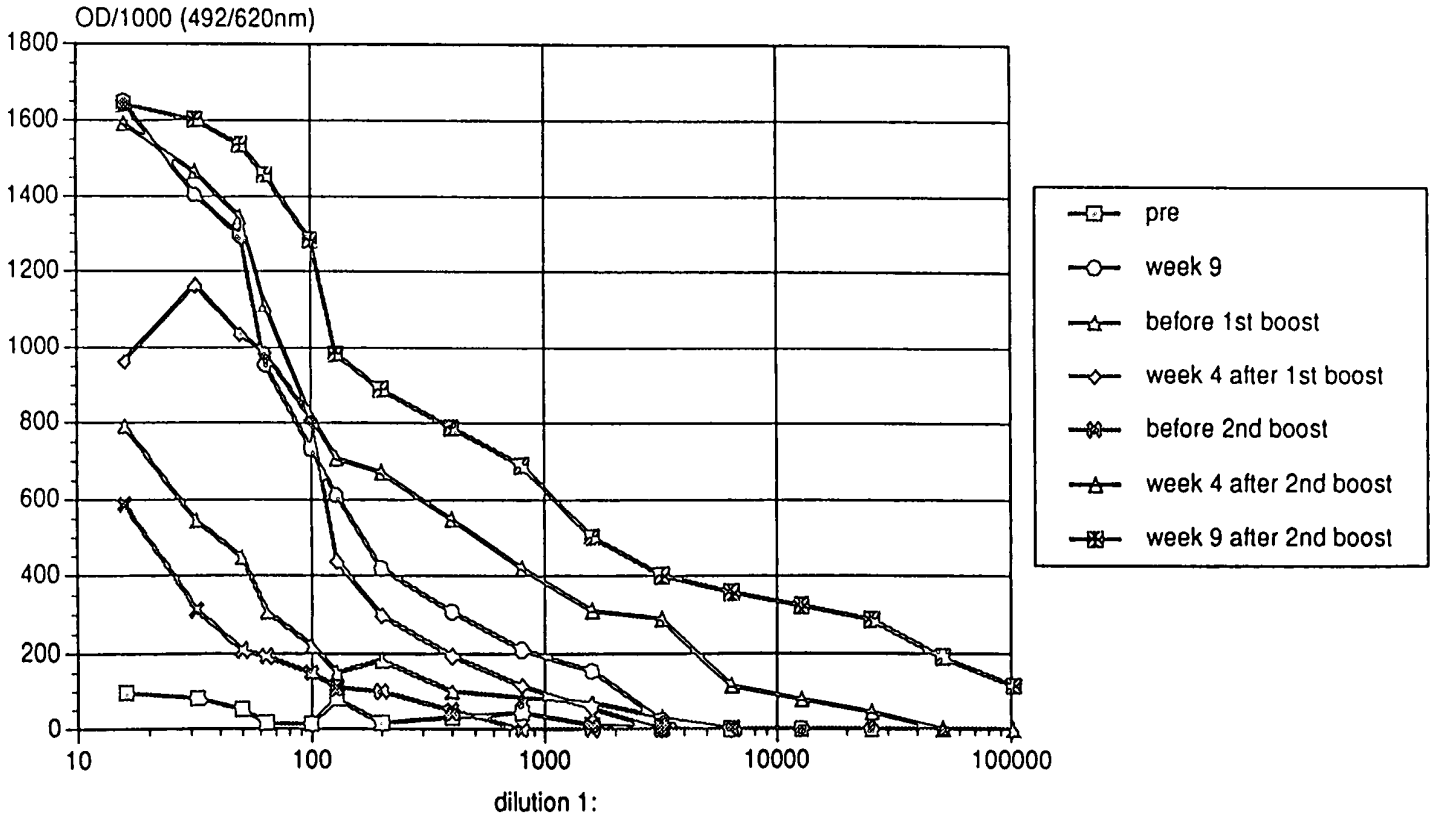
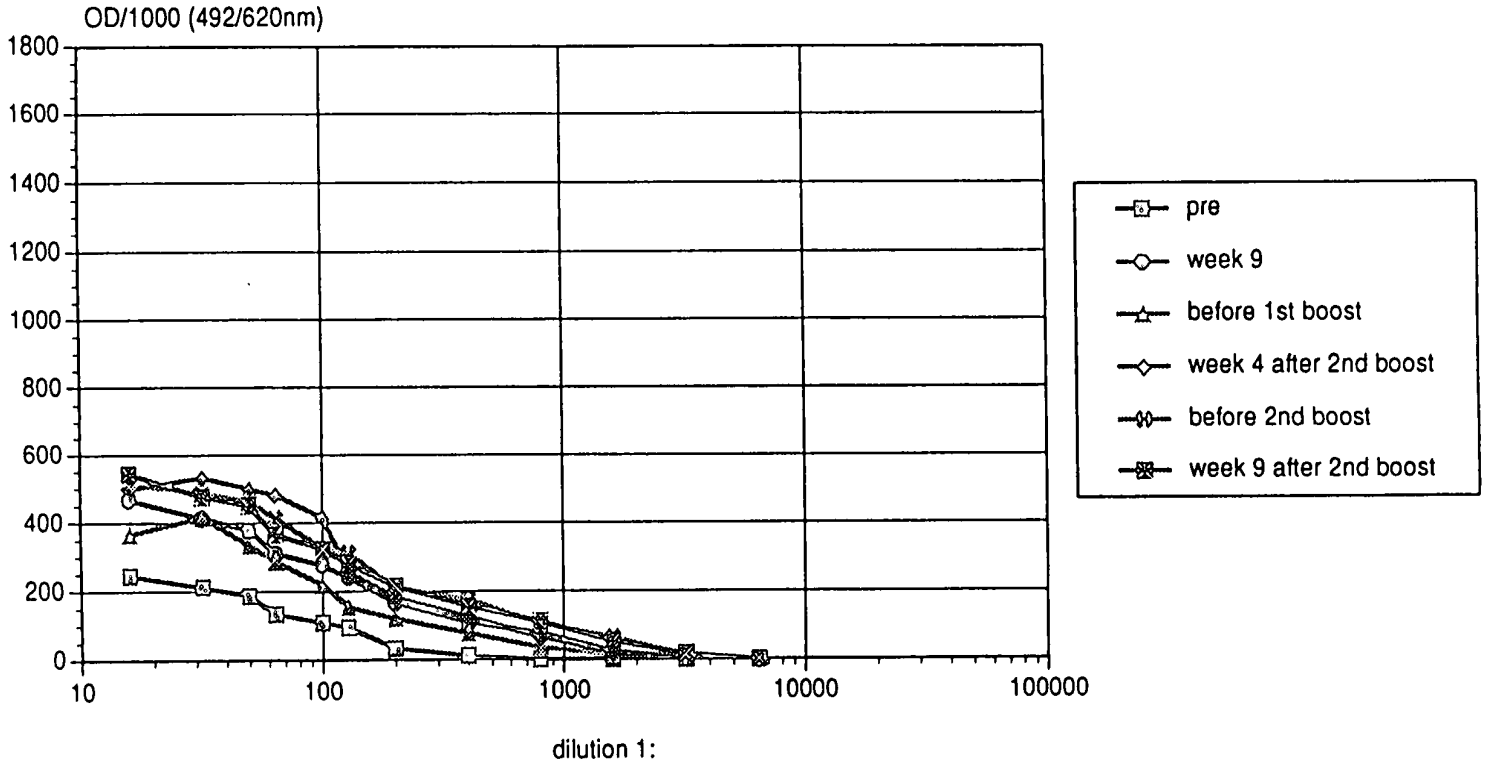


Figure 20

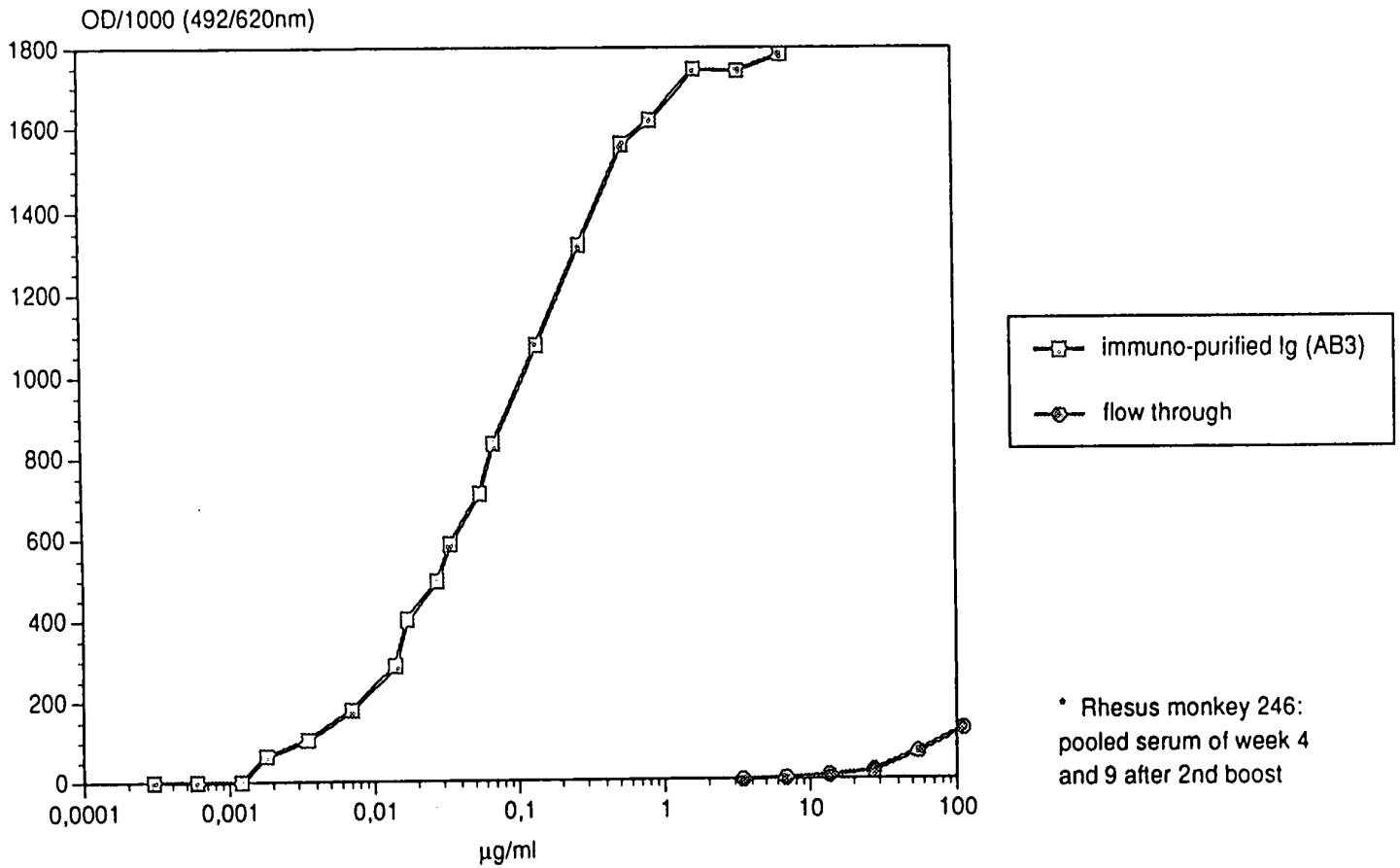
Immunization of monkey E23 with anti-id BR55-2#E4 Serum Ig binding to WM 9 melanoma cells (cell- ELISA)



ESSAGED ESSAGED

Figure 21

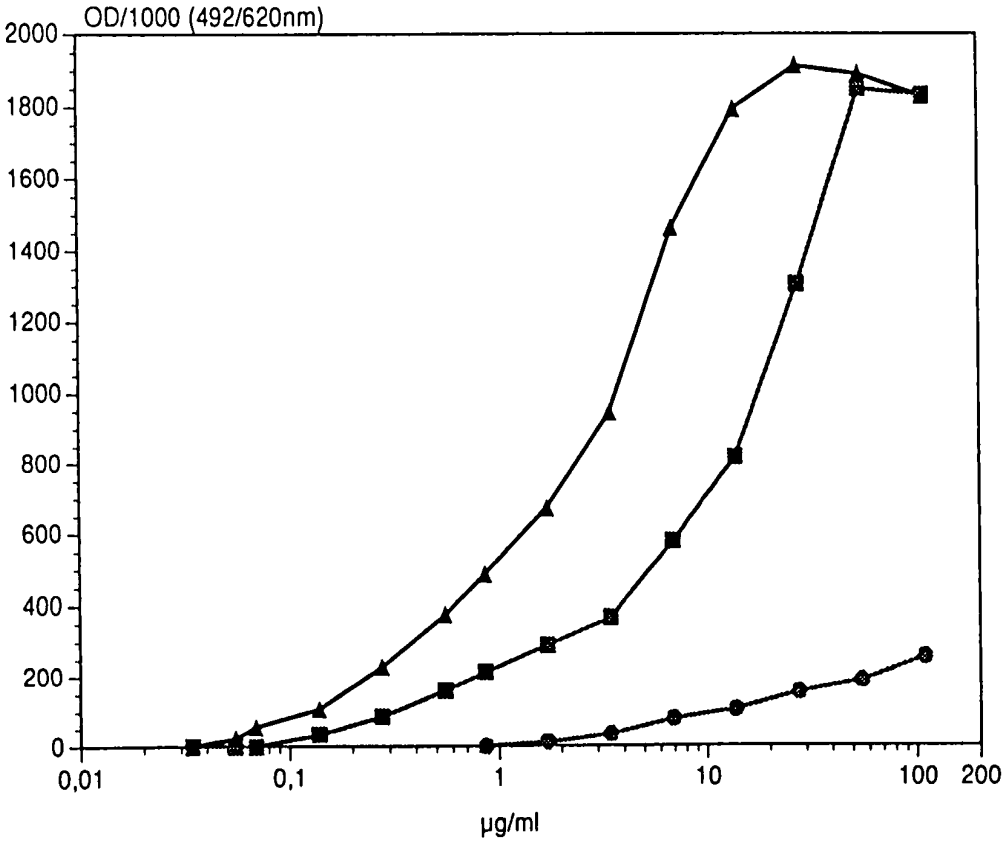
Rhesus monkey Ig immunopurified on anti-id BR55-2#E4*: binding to anti-id BR55-2#E4 (ELISA)



* Rhesus monkey 246: pooled serum of week 4 and 9 after 2nd boost

Figure 22

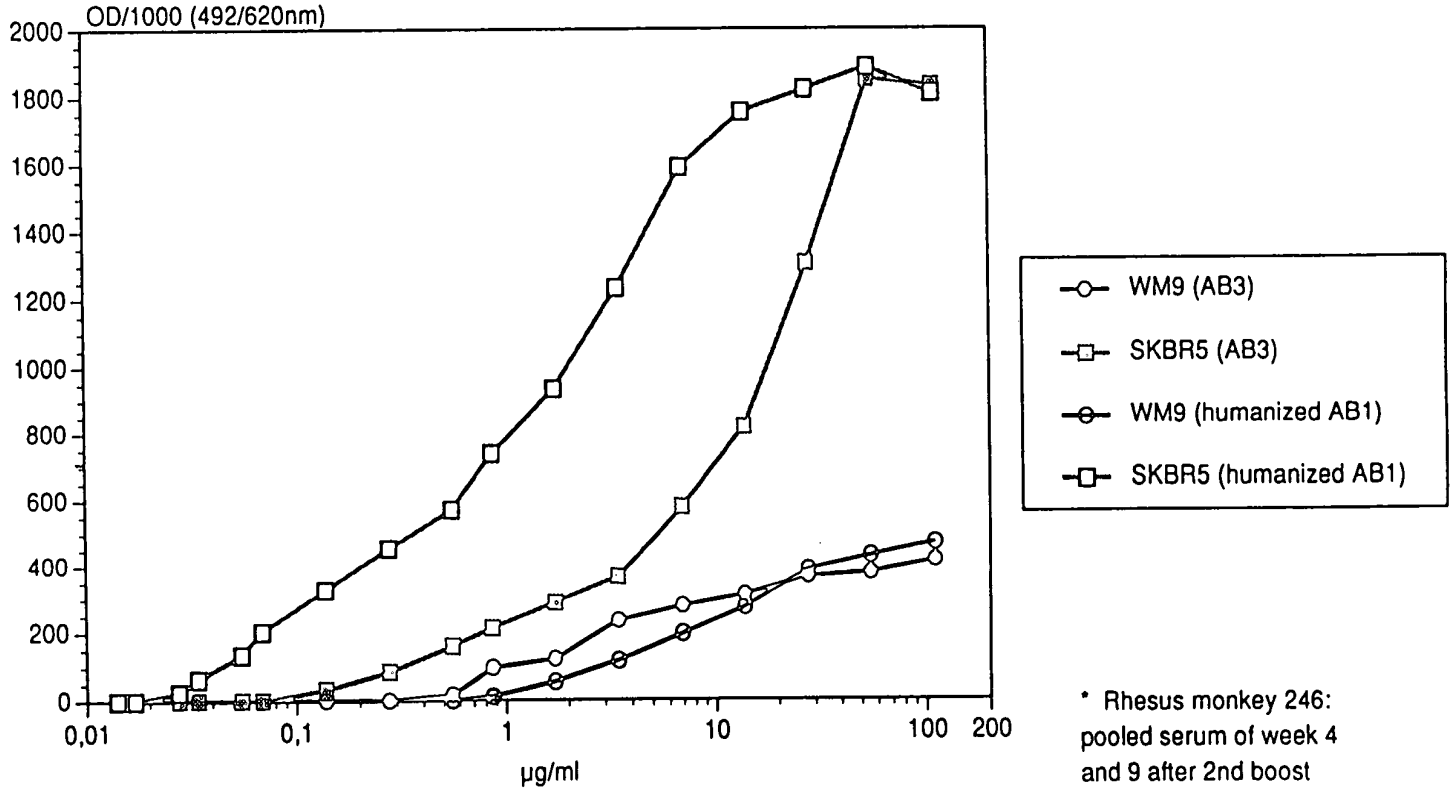
Rhesus monkey Ig immunopurified on anti-id BR55-2#E4*: binding to SKBR5 breast cancer cells (cell-ELISA)



* Rhesus monkey 246: pooled serum of week 4 and 9 after 2nd boost

Figure 23

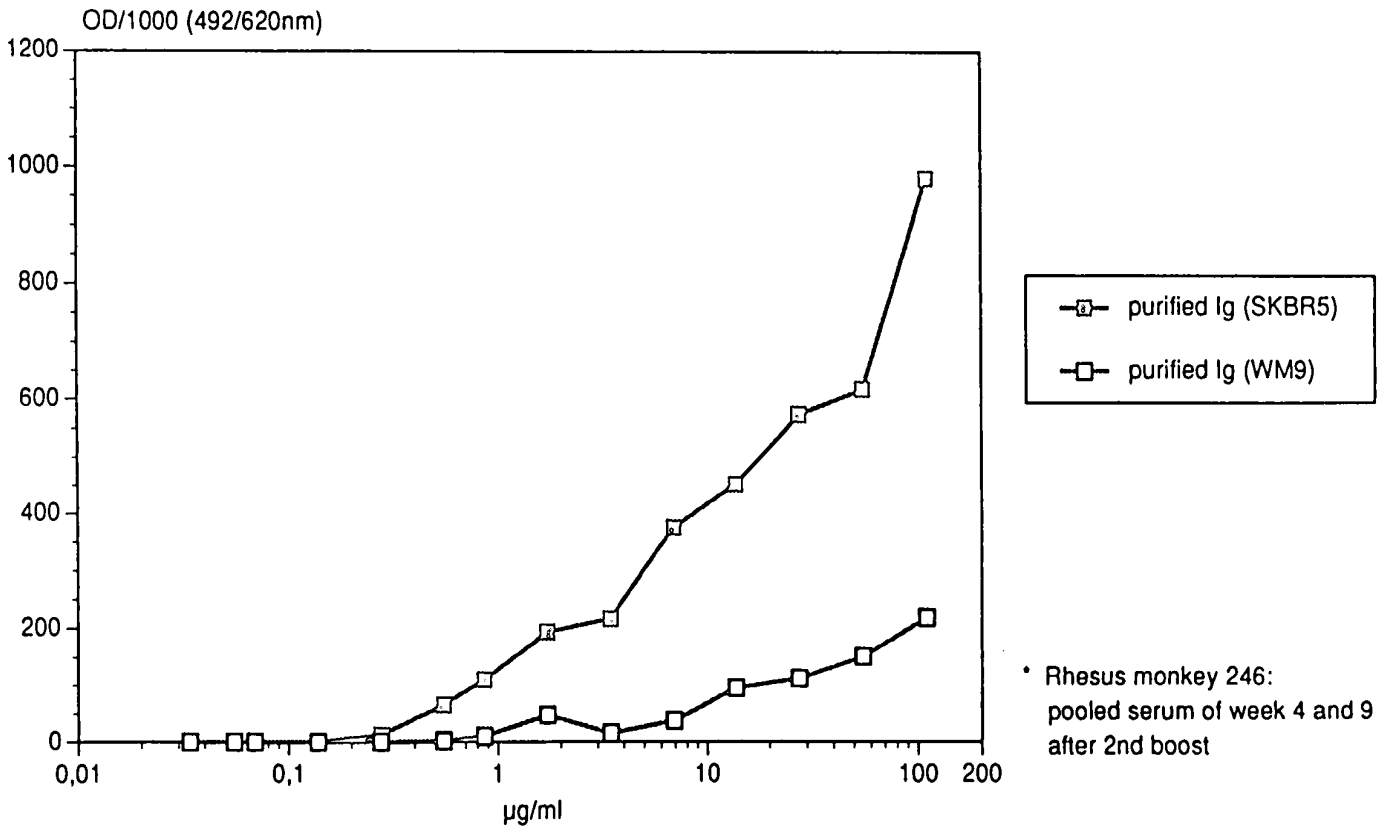
Rhesus monkey Ig immunopurified on anti-id BR55-2#E4*: binding to WM9 (melanoma) and SKBR5 (breast cancer) cells (cell-ELISA)



* Rhesus monkey 246: pooled serum of week 4 and 9 after 2nd boost

Figure 24

Rhesus monkey Ig immunopurified on anti-id BR55-2#E4*: binding to SKBR5 - and WM9 - cell membranes (ELISA)

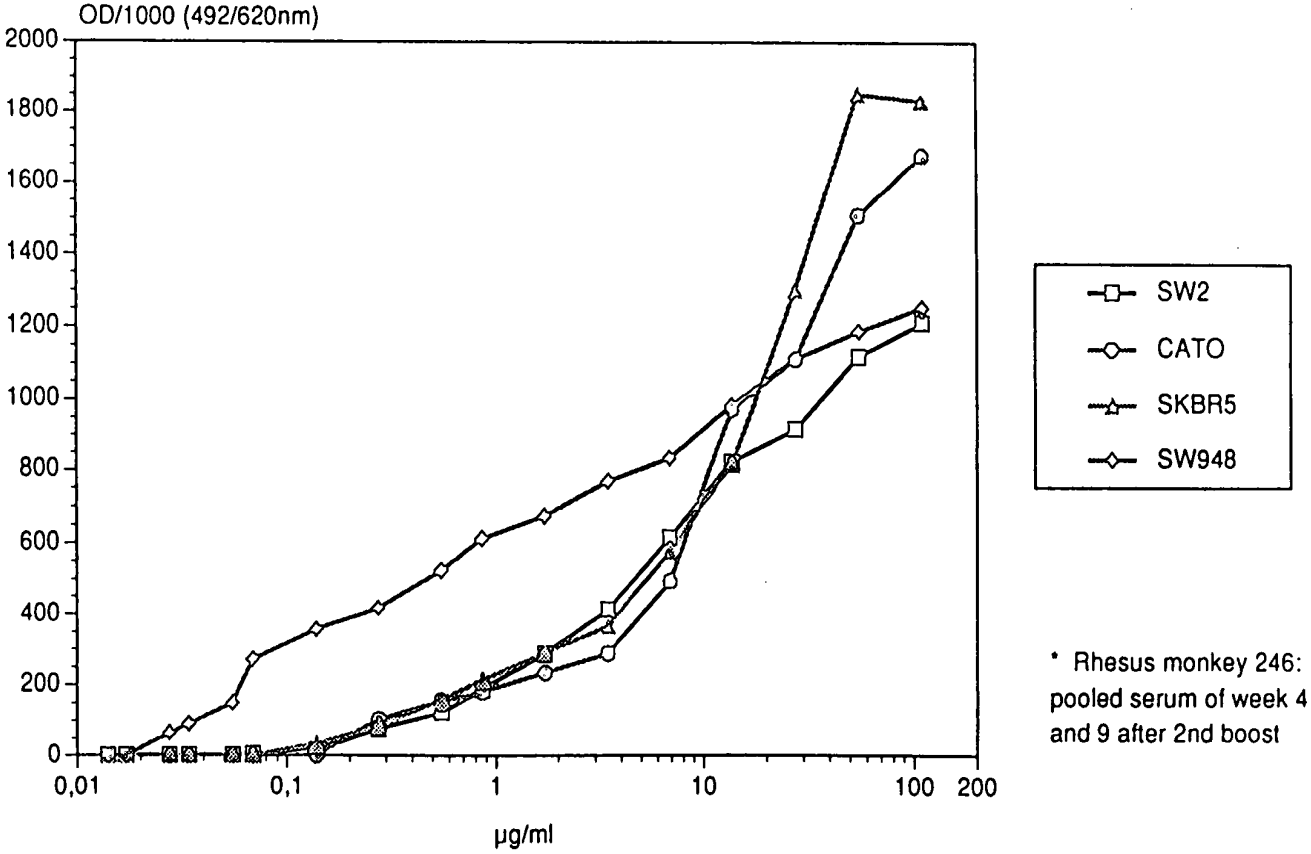


* Rhesus monkey 246: pooled serum of week 4 and 9 after 2nd boost

SUBSTITUTE SHEET

Figure 25

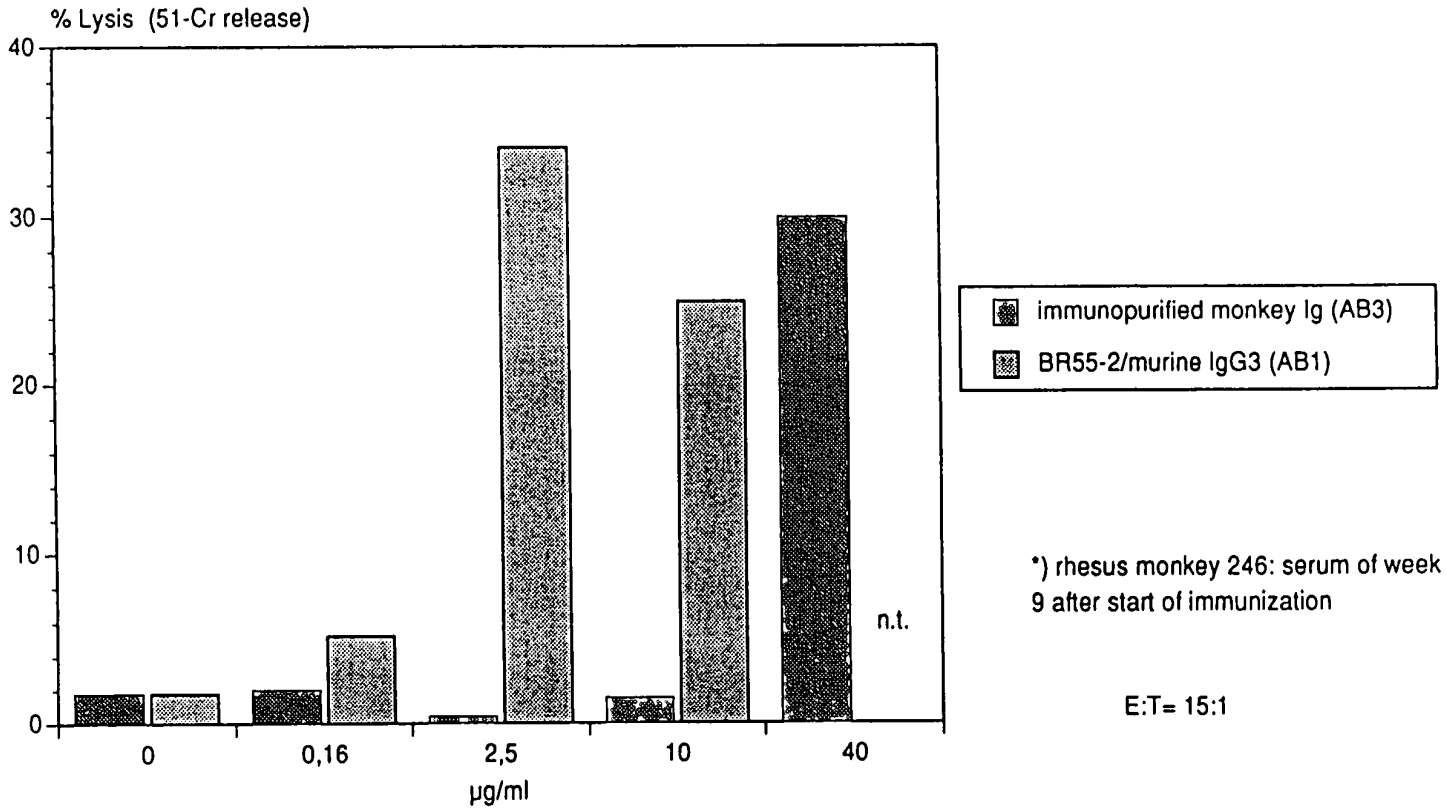
Rhesus monkey Ig immunopurified on anti-id BR55-2#E4*: binding to different cell lines (cell-ELISA)



* Rhesus monkey 246: pooled serum of week 4 and 9 after 2nd boost

Figure 26

ADCC to SKBR5 breast cancer cell line mediated by human PBMC and BR55-2/murine IgG3 or immunopurified Ig of a rhesus monkey* immunized with anti-id BR55-2#E4

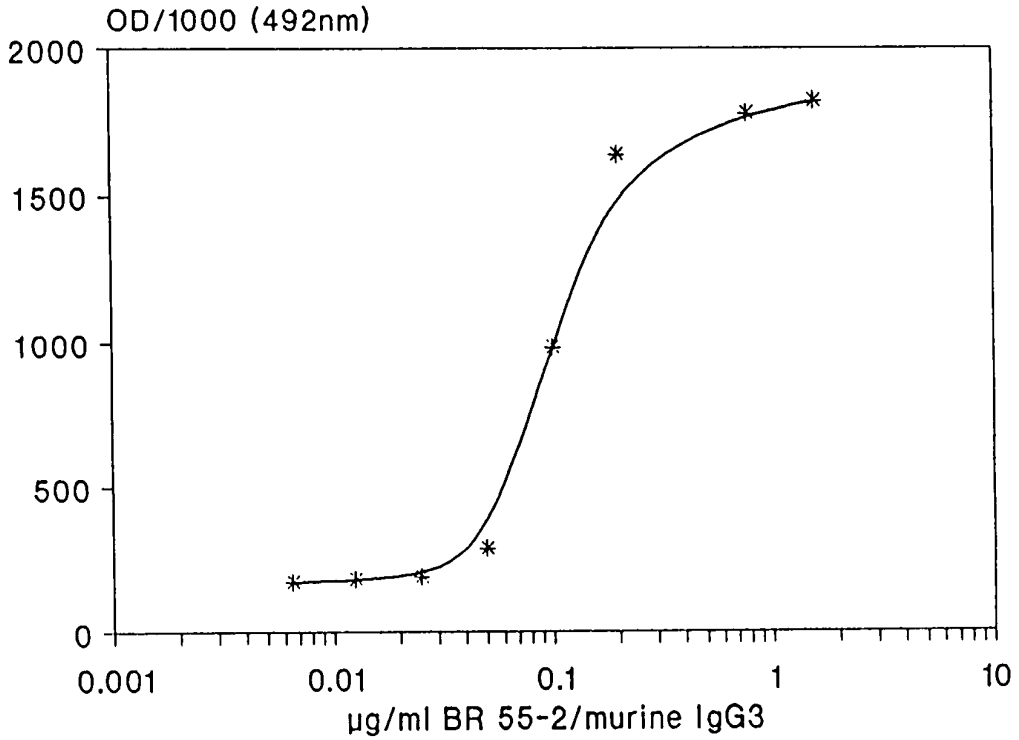


*) rhesus monkey 246: serum of week 9 after start of immunization

E:T= 15:1

Figure 27

ELISA for the determination of
BR 55-2/murine IgG3 in human serum
Standard curve



EP 0 590 058 B1

Figure 28

ELISA for the determination of BR 55-2/chimeric human IgG1 in human serum - standard curve

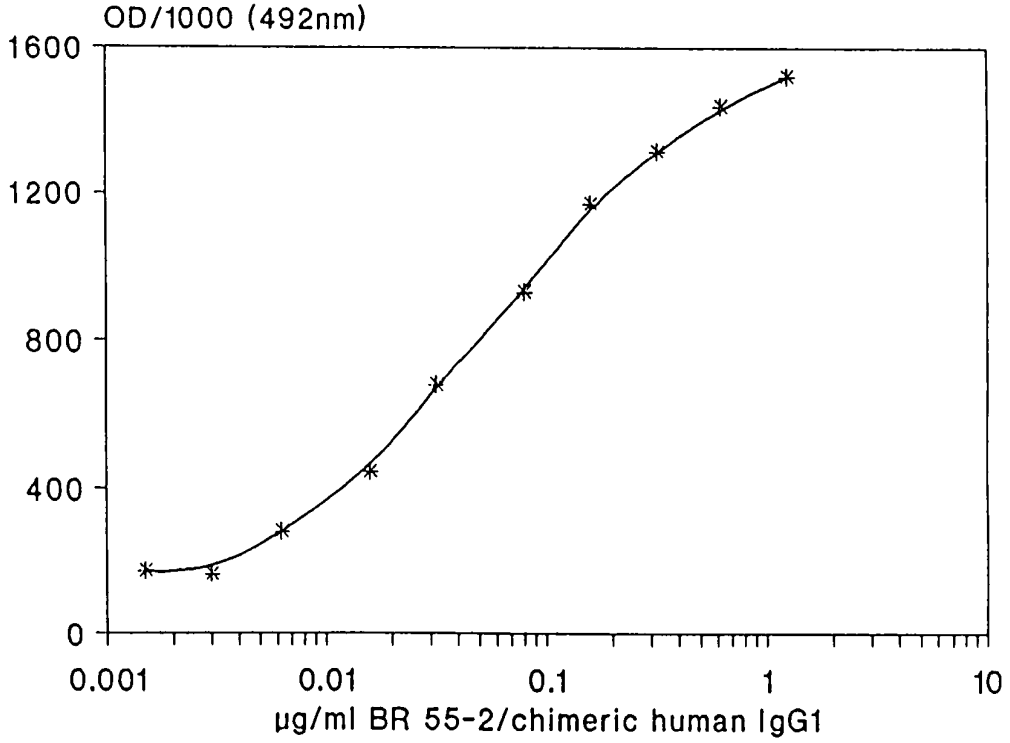


Figure 29

ELISA for the determination of BR 55-2/chimeric human IgG3 in human serum - standard curve

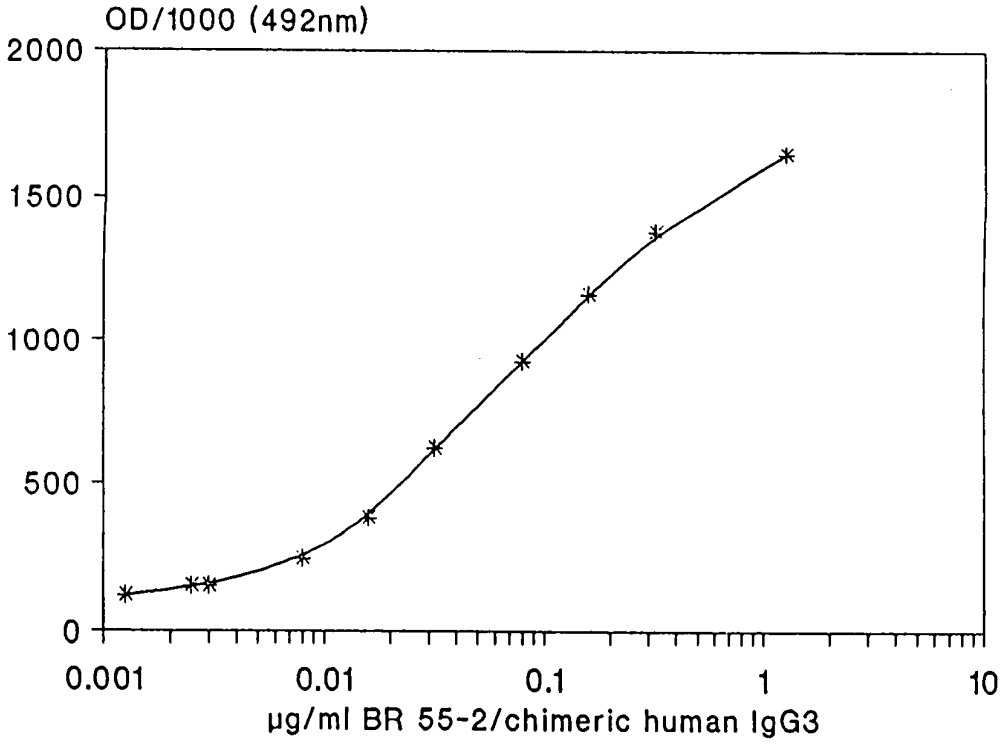


Figure 30

Binding of BR55-2/chimeric IgG1 and BR55-2/humanized IgG1 to anti-id BR55-2#E4

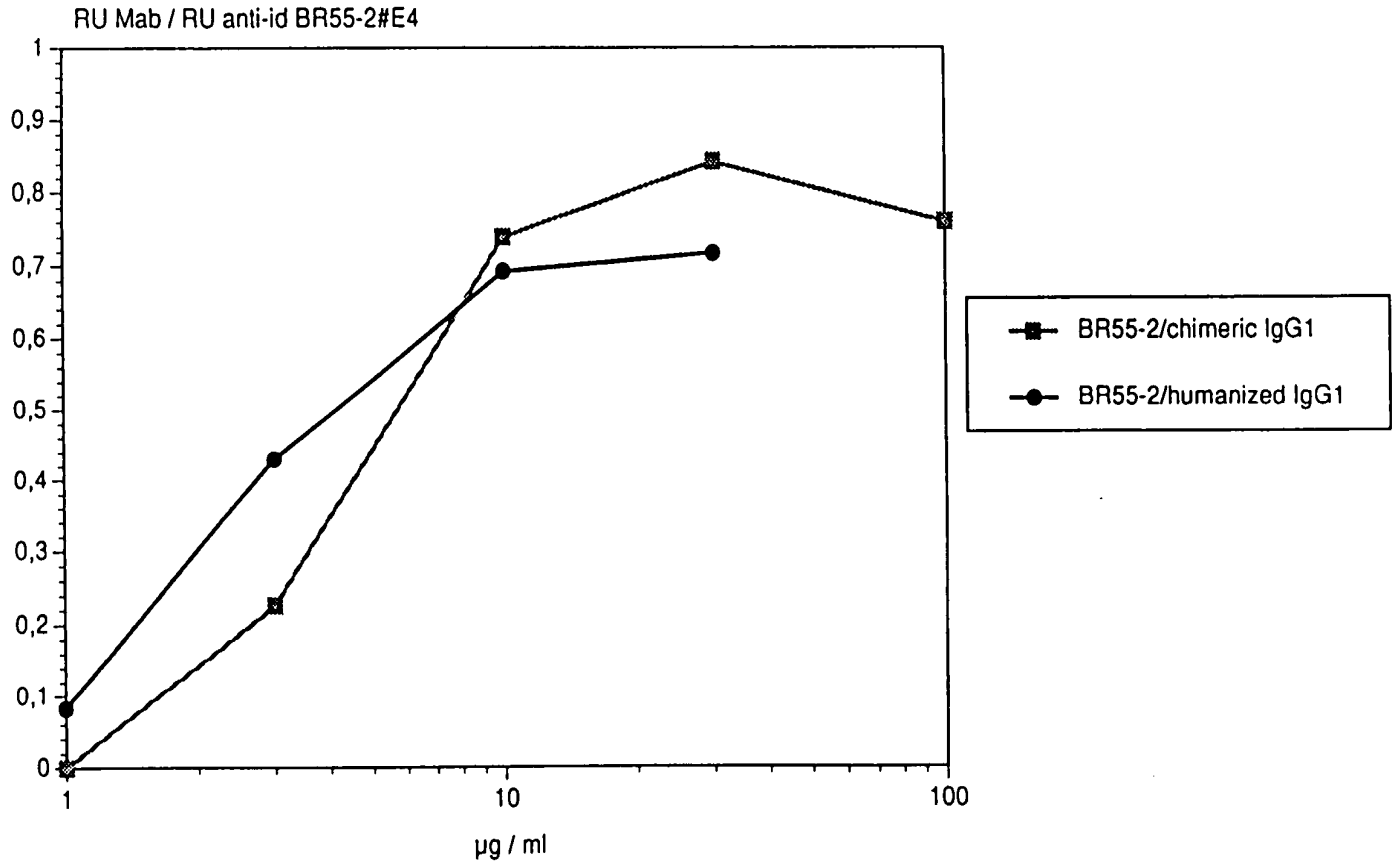


Figure 31

Binding of BR55-2/chimeric IgG1 and BR55-2/humanized IgG1 to anti-id BR55-2#C11

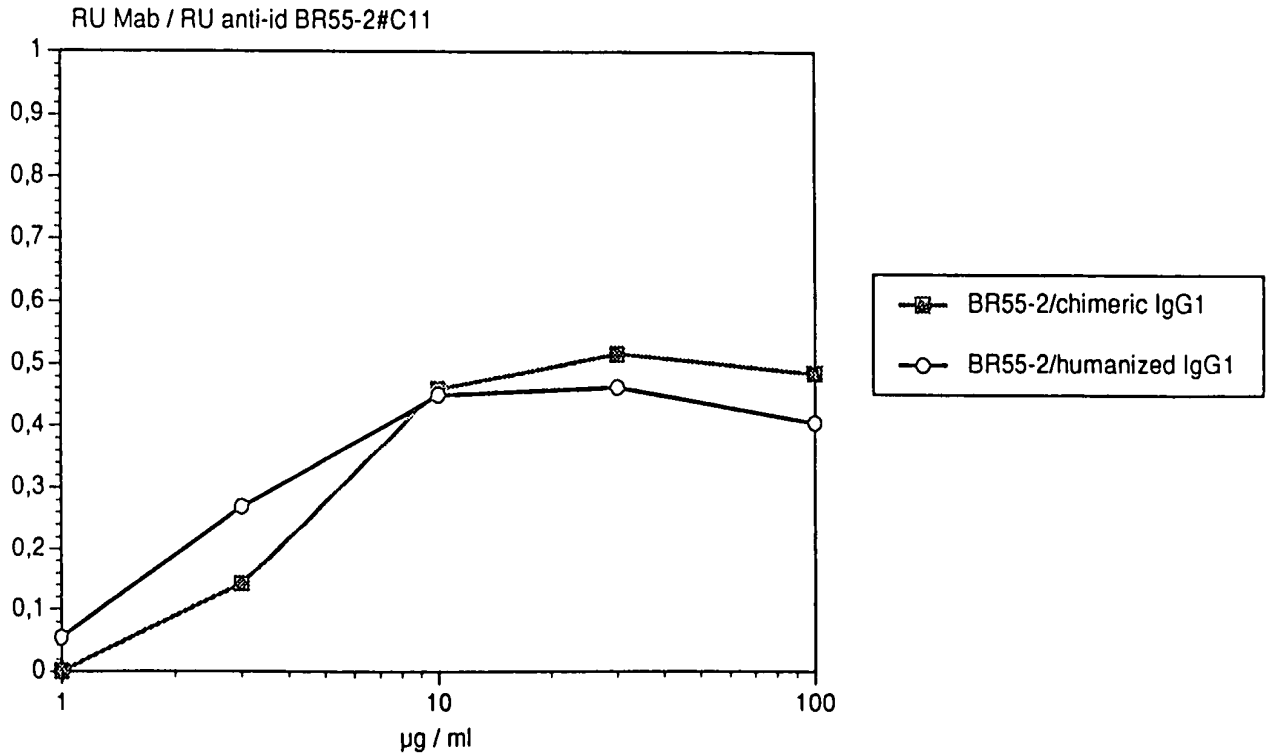


Figure 32

Binding of BR55-2/chimeric IgG1 and BR55-2/humanized IgG1 to anti-id BR55-2#B3

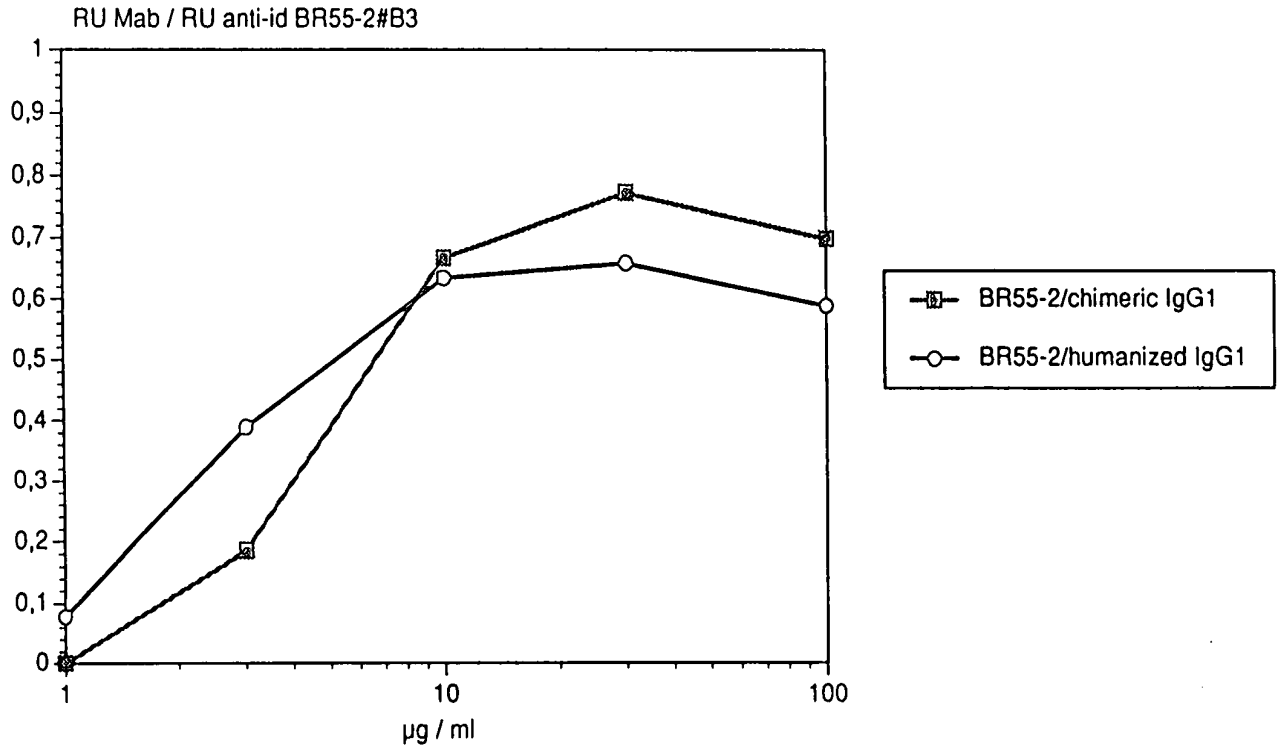


Figure 33

Binding of BR55-2/chimeric IgG1 and BR55-2/humanized IgG1 to anti-id BR55-2#B9

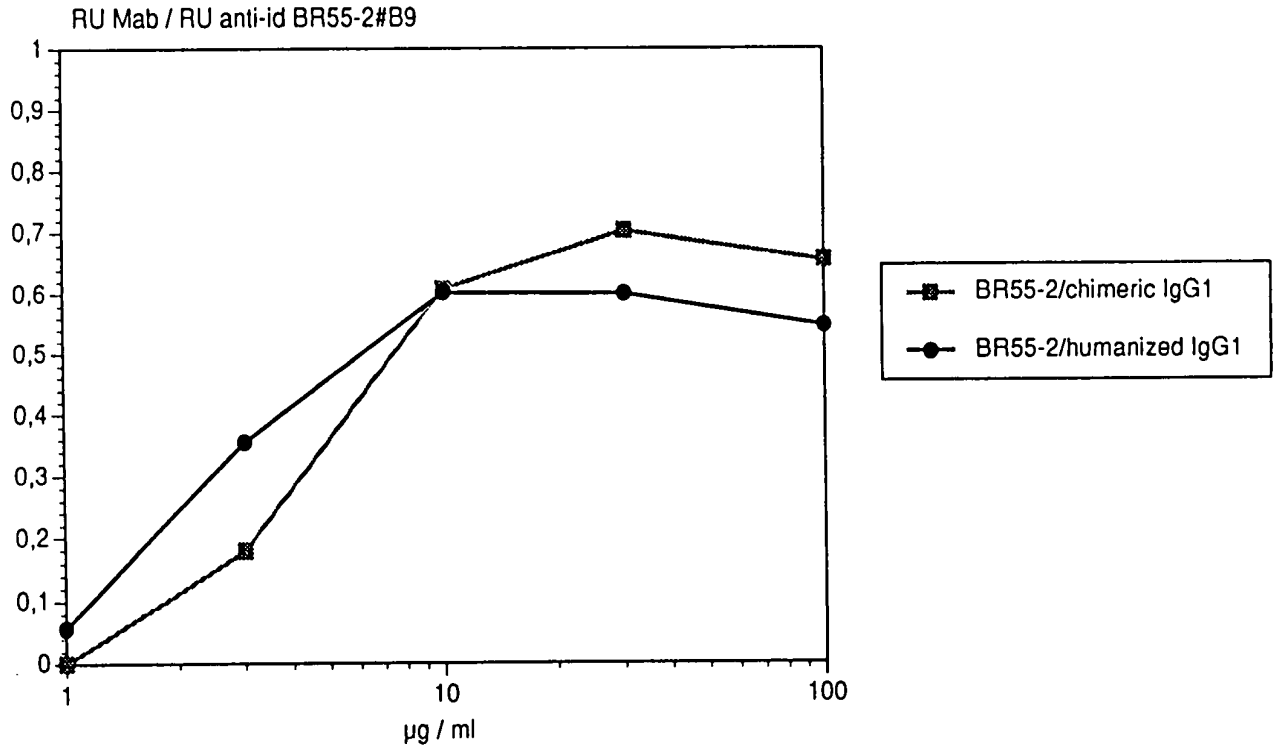


Figure 34

Binding of BR55-2/chimeric IgG1 and BR55-2/humanized IgG1 to anti-id BR55-2#G6

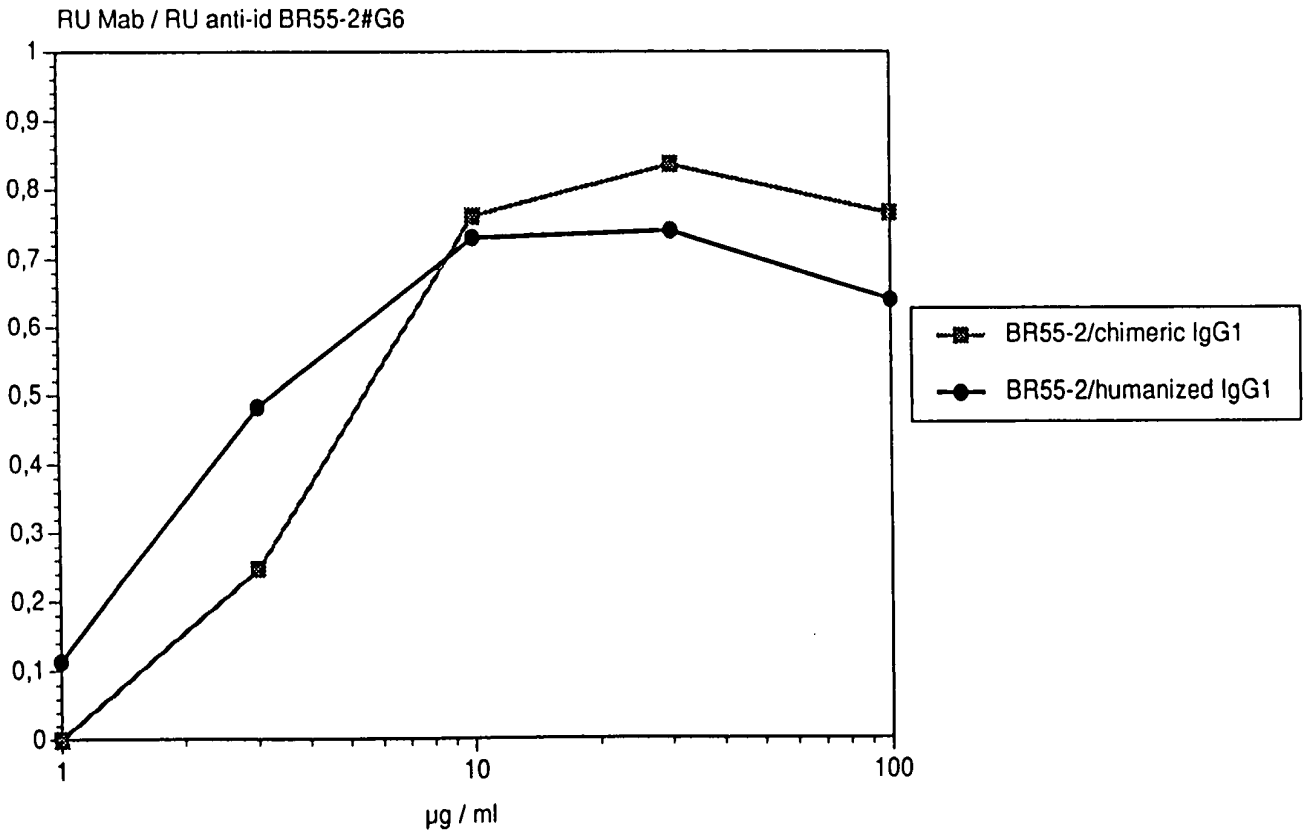
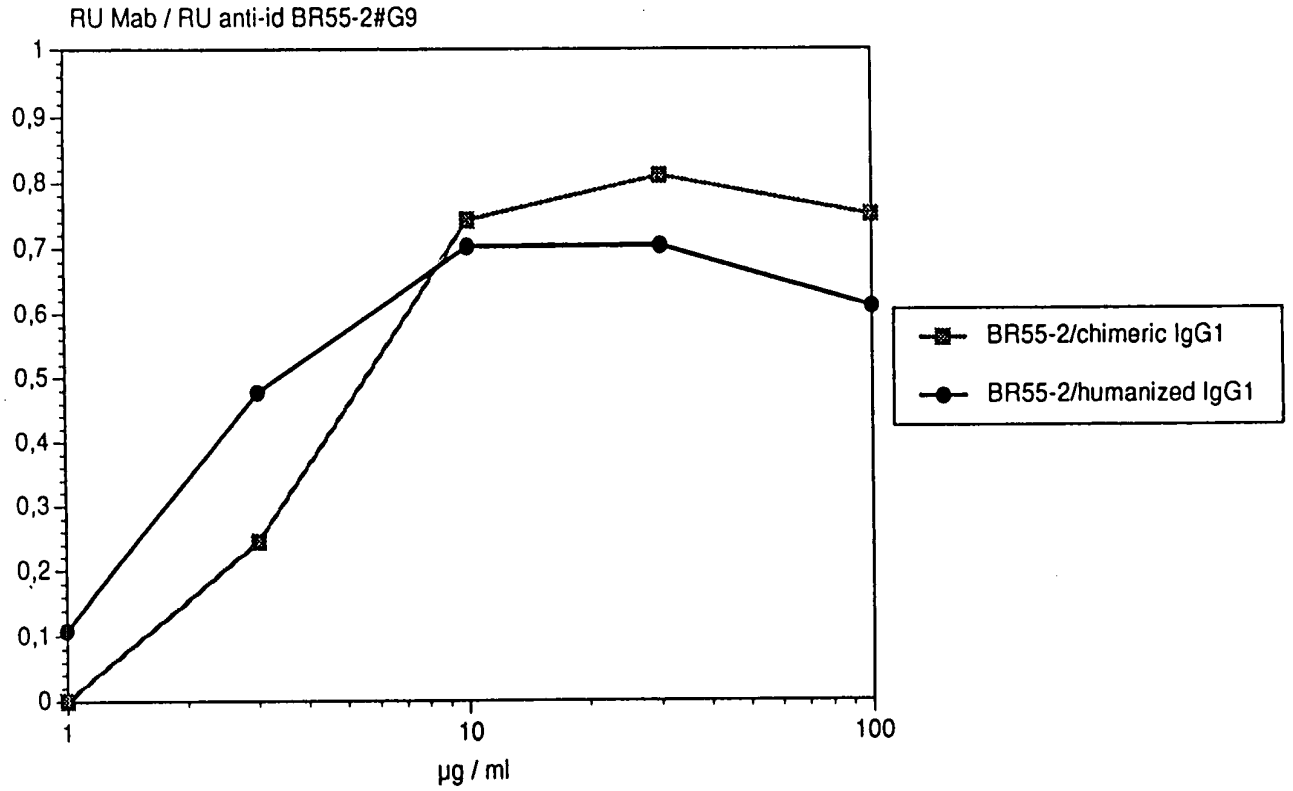


Figure 35

Binding of BR55-2/chimeric IgG1 and BR55-2/humanized IgG1 to anti-id BR55-2#G9



EDS0E0 E9900001

Figure 36

ELISA for the determination of BR 55-2/humanized IgG1 in human serum Standard curve

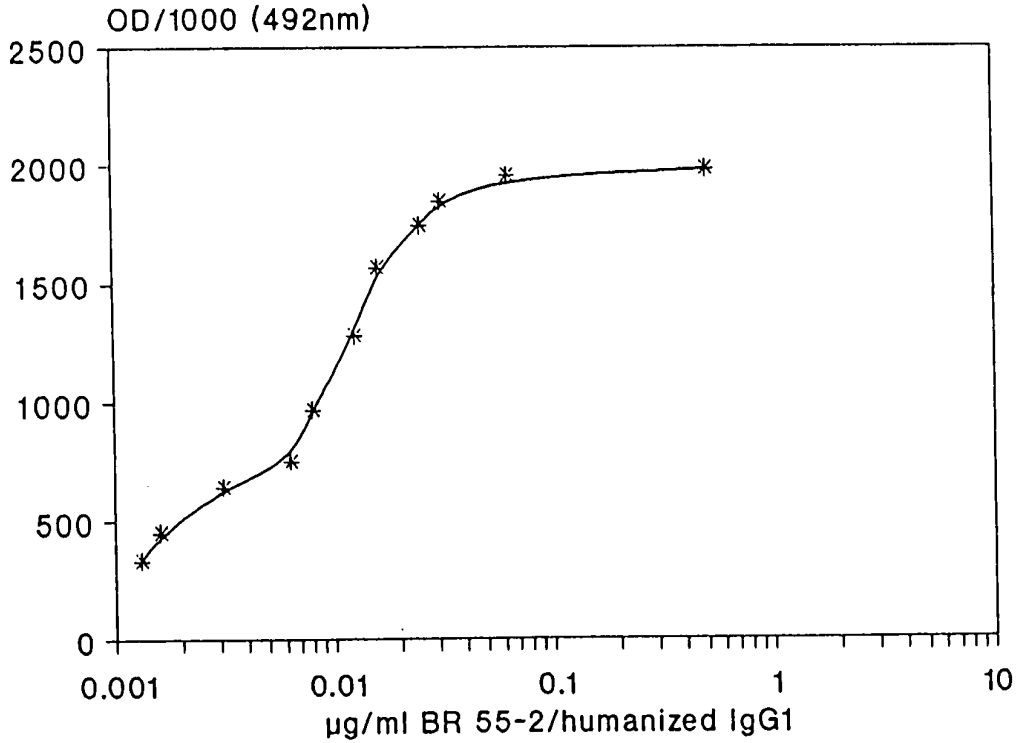


Figure 37

Binding competition of BR55-2/murineIgG3 to a-ID BR55-2 by BR55-2/chim.human IgG1 and BR55-2/chim.human IgG3

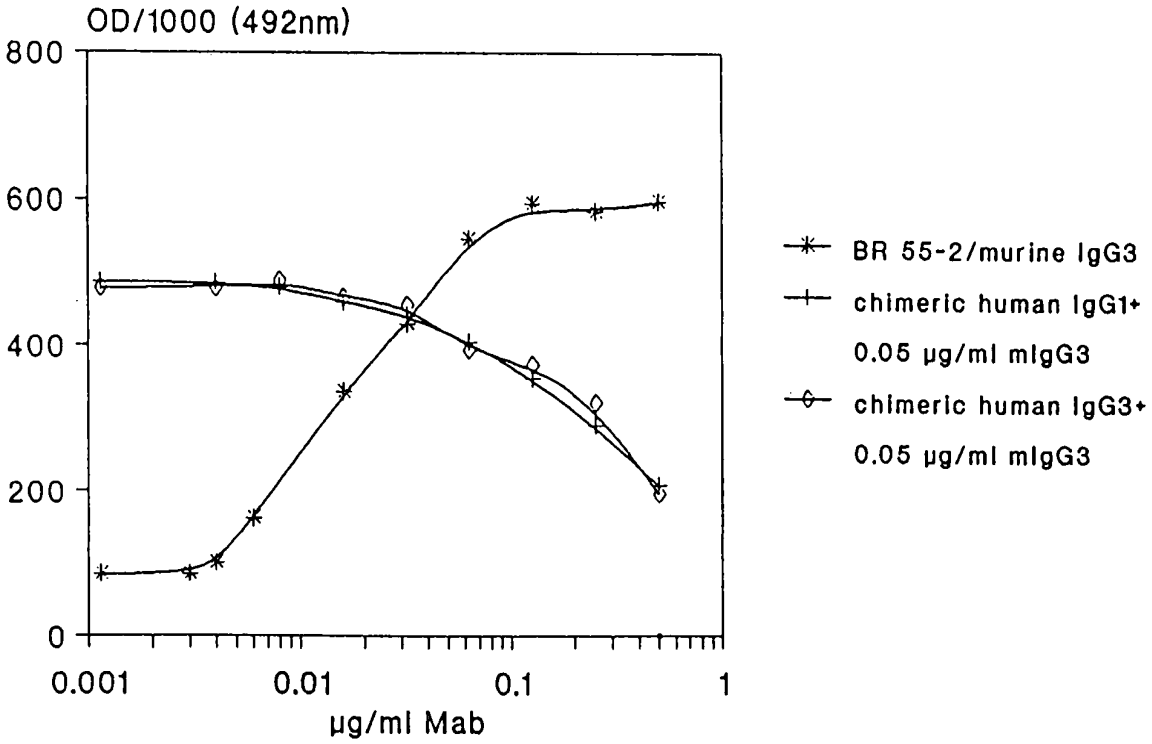
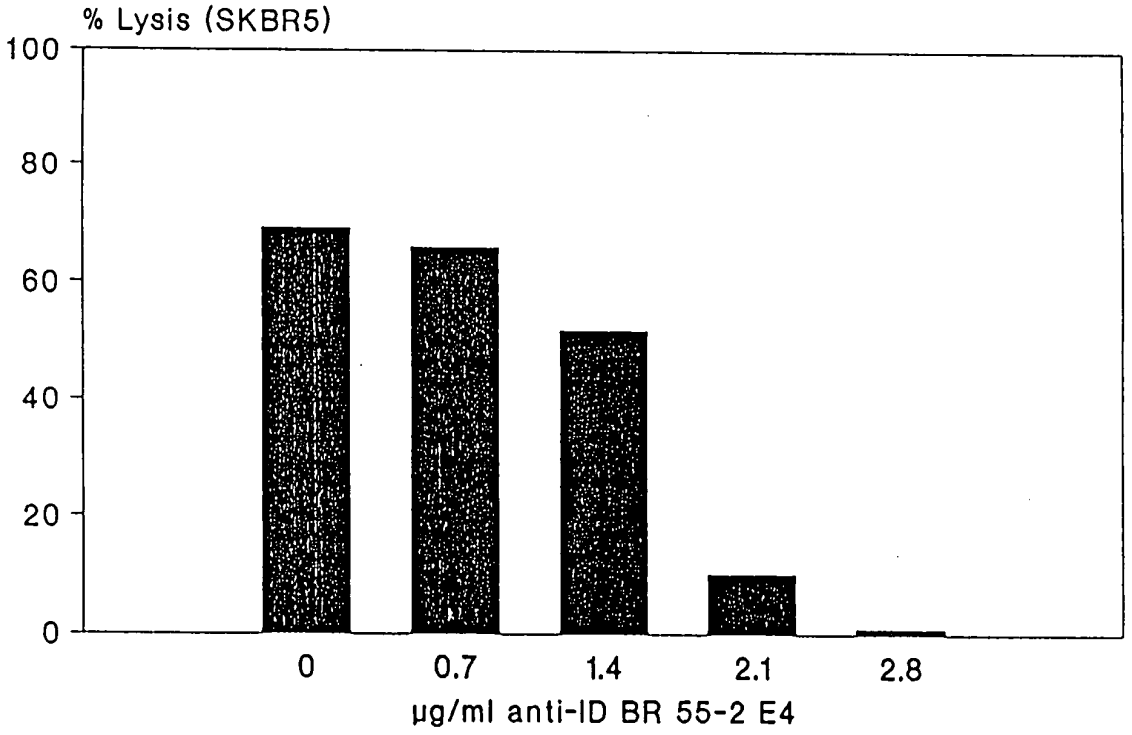


Figure 38

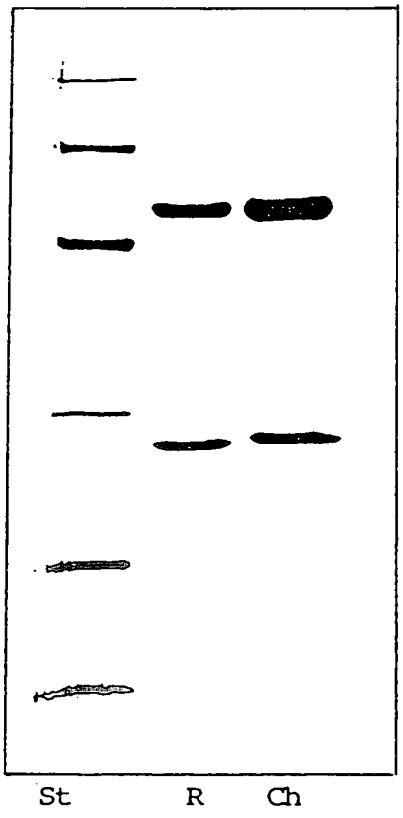
Complement dependent cytotoxicity mediated by BR 55-2/murine IgG3 Inhibition by anti-ID BR 55-2 E4



BR 55-2/murine IgG3: 1,4µg/ml

Figure 39

S D S - P A G E
Reducing conditions



St = Standards
R = Reference Mab
Ch = BR55-2/chimeric human IgG1